RAINDE REZENDE
**ASPECTS OF ORAL
IMPLANT TECHNOLOGY
AND OSSEOINTEGRATION**

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AND OSSEOINTEGRATION**

Malmö University
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To my beloved parents, precious brother,
and the love of my life, *Markus*

TABLE OF CONTENTS

THESIS AT A GLANCE	12
.....	13
ABSTRACT	14
LIST OF PAPERS	18
ABBREVIATIONS AND ACRONYMS	20
Abbreviations	20
Chemical element symbols	27
Gene and protein symbols	28
.....	29
INTRODUCTION	30
Titanium as a biomaterial	33
Osseointegration: on the evolution of modern implant dentistry	35
MECHANISMS OF HOST-TO-IMPLANT INTERACTIONS....	39
Bone architecture and structural composition	39
Bone mechanical properties	44
Cellular components of bone tissue	45
Wound-healing phenomena: cell-to-cell communication and healing pathways	49
Prognostic factors and long-term maintenance of the peri- implant bone support	64
STRATEGIES TO ENHANCE BONE DEPOSITION AND SHORTEN HEALING TIMES	68
Implant surface modifications	69
Topographical properties	69
Physical and chemical properties	71
Additive vs. subtractive manufacturing of oral implants	75

Chemically modified hydrophilic implant surfaces	78
Implant macrodesign	82
Primary implant stability and micromotion	82
Implant body, length, and diameter	84
Thread-shape designs	86
Surgical preparation of the implant site: the drilling technique.....	90
Osseodensification vs. conventional implant subtractive drilling.....	91
Osteocompression and biomechanical strain	95
Oversized preparation of the implant site.....	98
The hybrid healing pathway: bringing together interfacial remodeling and intramembranous-like bone healing mode	101
DISSERTATION'S RATIONALE.....	104
AIMS	106
MATERIALS AND METHODS.....	108
Experimental units and study design	108
Titanium implants.....	108
Titanium disks	109
Surface topographical and physico-chemical properties	110
Preclinical <i>in vitro</i> analyses	111
Mouse monocyte/macrophage cell line (RAW264.7)	112
Cell expansion, seeding, and differentiation to osteoclasts on titanium disks	113
Osteoclast differentiation and viability	115
Gene expression analysis: reverse transcriptase real- time quantitative polymerase chain reaction (RT-qPCR)....	116
Qualitative and quantitative image analyses of osteoclast proliferation, adhesion, and morphology.....	123
Systematic review and meta-analysis	126
PICO question and inclusion criteria.....	127
Search strategy, screening methods, and study selection	128
Data extraction	128
Risk of bias and methodological quality assessment	129
Preclinical <i>in vivo</i> analysis.....	131

Experimental animal models and study design	131
Ethical approvals	132
Surgical procedures	133
Post-operative care.....	138
Ex vivo analytical procedures and methods	139
Non-decalcified histotechnical procedures.....	139
Histomorphometrical analysis of bone-implant interface: parameters assessed.....	140
Assessment of interfacial biomechanical properties of osseointegrated implants: parameters assessed	142
Statistical analysis	144
Meta-analysis.....	147
SUMMARY OF RESULTS.....	149
The effect of hydrophilicity on early osseointegration and biomechanical properties of titanium implants <i>in vivo</i> (Study I & II)	149
Descriptive histological results	149
Histomorphometric results	151
Biomechanical results	153
.....	154
The effect of hydrophilicity and implant macrodesign on osteoclast differentiation, activation, and survival in a murine macrophage-like cell model (Study III)	155
Osteoclast differentiation and cell viability	155
Osteoclast-specific gene expression	157
Osteoclast proliferation, adhesion, and morphology	157
The effect of oversized surgical preparation of the implant site on osseointegration of titanium implants in experimental animal models (Study IV & V).....	162
Systematic review: study selection and characteristics	162
Descriptive histological results	164
.....	166
Metanalysis of preclinical <i>in vivo</i> studies	167
Histomorphometric results	170
DISCUSSION.....	172
Rational for performing preclinical <i>in vitro</i> and <i>in vivo</i> testing of biomaterials	172
<i>In vitro</i> models to study biomaterial-based modulation	

of cellular and molecular mechanisms	172
<i>In vivo</i> models to study tissue response and implant integration	174
The effect of hydrophilicity on osseointegration and biomechanical properties of titanium implants	177
Biological aspects	178
Biomechanical aspects	180
The potential of hydrophilicity to positively modulate the early inflammatory response and its effect on osteoclast differentiation, activation, and survival	181
The effect of oversized surgical preparation of the implant site on osseointegration and marginal bone level	184
Biomechanical aspects	185
Biological aspects	187
CONCLUSIONS AND FUTURE PERSPECTIVES	192
POPULÄRTVETENSKAPLIG SAMMANFATTNING	195
ACKNOWLEDGEMENTS	198
REFERENCES	204

THESIS AT A GLANCE

Study	Aim	Illustration	Key findings
<p>I</p> <p>Histomorphometric evaluation of a dual acid-etched vs. a chemically modified dual acid-etched implant surface. An experimental study in dogs.</p>	<p>To evaluate the histomorphometrical parameters (mineralized bone-to-implant contact [BIC] and relative peri-implant bone density [BD]) at implants with a sandblasted, dual acid-etching, with or without an additional chemical modification (hydrophilic SAE-HD and hydrophobic SAE, respectively) at early stages of osseointegration in the tibia of Beagle dogs.</p>		<p>Histomorphometrical parameters increased with time at SAE-HD and SAE implants, and no significant differences were observed between the two groups for any of the evaluated parameters and at any observation time-point during the early osseointegration period.</p>
<p>II</p> <p>Interfacial biomechanical properties of a dual acid-etched versus a chemically modified hydrophilic dual acid-etched implant surface: an experimental study in Beagles.</p>	<p>To evaluate the biomechanical properties (removal torque value [RTV] and a complete set of intrinsic properties [removal energy and connection stiffness]) at SAE-HD and SAE implants at the early stages of osseointegration in the tibia of Beagle dogs.</p>		<p>In terms of biomechanical properties, no significant differences were observed between SAE-HD and SAE implants for any of the evaluated parameters and at any observation time-point but a slight increase over time during the early osseointegration period.</p>

ABSTRACT

Implant rehabilitation of edentulous patients is a predictable treatment with high long-term survival rates (90-95%) reported for the major dental implant systems after 5-10 years in function. Throughout recent years, different materials, implant surface technologies, macrodesigns, and surgical techniques have been developed to enhance osseointegration and lessen treatment timeframes, for example allowing immediate or early functional loading in patients with reduced bone density.

In regards with implant surface properties, hydrophilicity ensures a more rapid coverage of the implant surface with blood and biological fluids, and a faster blood clot formation; this, in combination with the synergistic effect of micro- and/or nanotopography of the implant surface, lead to faster resolution of inflammation and positive modulation of osteogenesis, in turn enhancing the rate and quality of bone-to-implant contact (BIC). Specifically, a novel modification of sandblasted, dual acid-etched surface (SAE) implants to increase hydrophilicity (SAE-HD) has been suggested to enhance osseointegration during the early healing period in a pre-clinical *in vivo* model of rabbit tibia in comparison to the hydrophobic SAE surface. However, its histomorphometrical parameters of osseointegration and biomechanical properties have not been investigated in large animal platforms of low- and high-density bone. Furthermore, there is limited knowledge regarding the possible combined modulatory impact of implant macrogeometry and surface hydrophilicity on osteoclastogenesis.

In regards with the surgical technique for implant installation, contemporary practice dictates that the implant site is prepared slightly narrower than the implant diameter as per standard (i.e. there is slight mismatch of the inner and outer implant diameter in relation to the drilled bone walls with the implant threads engaging with the bone in the pursuit of high primary implant stability, as measured by maximum insertion torque values (ITV). However,

excessive lateral compression of highly-dense trabecular bone and thick cortex has been shown to cause microdamage and necrosis, triggering extensive interfacial bone remodeling, which in turn negatively affects the biomechanical competence of the implant site throughout the healing period.

Recently, an oversized drilling technique has been proposed, where the final drill for site preparation has similar or slightly larger diameter than that of the inner implant diameter; this creates closed “healing chambers” between the inner implant diameter and the surgically instrumented bone wall. It is suggested that in spite of low ITV achieved with this surgical approach, the osteoconductive properties of modified implant surfaces positively modulate host-to-implant interactions and enhance osseointegration. However, research to date appears remarkably controversial on this topic, while studies reporting on marginal peri-implant bone levels (MBL) of highly dense bone are limited.

The general aim of the present thesis was to evaluate the possible impact of aspects of current oral implant technology, i.e. surface hydrophilicity, implant macrogeometry, and surgical preparation of the implant recipient site, on implant osseointegration. Specifically, it was hypothesized that:

- Implants with a chemically modified micro-rough surface would achieve higher BIC and interfacial shear strength in comparison to hydrophobic implants.
- Oversized implant recipient sites, by applying sequential profiling and/or tapping of the bone, would promote higher amount and better quality of osseointegration in comparison to undersized-drilled sites.

In study I and II, histomorphometrical parameters (BIC and relative peri-implant bone density [BD]) and biomechanical properties (removal torque value [RTV], removal energy, and connection stiffness) at SAE (control) and SAE-HD (test) implants were assessed at early stages of osseointegration. Two pairs of SAE and SAE-HD implants, of same microgeometry, were installed bilaterally in the proximal tibia of six Beagle dogs and assessed after 2 and 4 weeks of healing. In study I, histomorphometrical parameters were analyzed on non-decalcified sections. In study II, the removal

torque test (RTQ) was conducted on a Shimadzu universal testing machine. Results showed that BIC, BD, RTV, and removal energy increased with time in both SAE and SAE-HD implants. No significant differences were observed between the two groups for any of the evaluated parameters and at any observation time-point but a slight increase over time during the early osseointegration period.

In study III, the influence of the implant thread design (trapezoidal vs. triangular threads) and surface hydrophilicity (SAE-HD vs. SAE) on osteoclast (OC) differentiation, activation, and survival was evaluated *in vitro* in a model of RAW 264.7 cells. Titanium disks with SAE or SAE-HD surface and different macrodesign, comprising of trapezoidal (HLX) or triangular threads (TMX) were used: HLX/SAE-HD, HLX-SAE, TMX/SAE-HD, and TMX/SAE. RAW264.7 macrophages were seeded on the disks, differentiated to OC by RANKL treatment and cultured for 5 days. OC differentiated on polystyrene plates were used as positive controls [CCPC (+)]. OC differentiation and viability were assessed by enzymatic TRAP activity and MTT assays. Expression levels of various OC-related genes were measured at the mRNA level by qPCR. SAE-HD surfaces negatively modulate macrophage/osteoclast viability. Specifically, SAE-HD with triangular threads increases the cellular pro-inflammatory status, while surface hydrophilicity and macrodesign do not seem to have a distinct impact on OC differentiation and activation *in vitro* in a model of RAW 264.7 cells.

In study IV, the current evidence on the effects of oversized surgical preparation of the implant site in terms of biomechanical properties (ISQ, ITV, and RTV) and biological parameters of osseointegration (BIC and BD) was systematically assessed in preclinical animal studies. Electronic databases were searched for preclinical *in vivo* controlled studies reporting on drilling techniques according to the relative implant-final drill discrepancy (IDD; mm), defined herein as the dimensional mismatch between the outer implant diameter and the bone wall in the implantation bed: (1) control ≥ 0.2 mm (press-fit/standard to undersized implant drilling technique); (2) test OD = 0.0 – 0.1 mm (oversized drilling technique to achieve either nearly or precisely similar dimension between the bone defect and the implant outer diameter); and (3) test

$GAP \leq -0.1$ mm (oversized drilling technique presenting a gap between the bone defect and the implant outer diameter). Random effects meta-analyses were performed for the outcomes ITV, RTV, BIC, and BD at different healing periods. Evidence from 12 studies indicate that oversized surgical preparation of the implant site appears to minimize marginal bone resorption characterized by fast rates of new bone formation in the healing chambers through an intramembranous-type healing mode. However, in terms of biological and biomechanical parameters of osseointegration, oversized implant sites does not seem to provide any additional benefit compared to narrower implant sites, neither at the time of implant placement nor during the early healing period, yielding comparable results at the late post-operative period in different experimental animal models.

In study V, the effect of oversized surgical preparation of the implant site on histomorphometrical parameters of osseointegration (BIC and BD) and marginal peri-implant bone level (MBL) of tapered implants with a progressive thread geometry was evaluated following 12 weeks *in vivo* in the mandible of minipigs. Ten implants with hydrophilic surface (SAE-HD) were inserted in the edentulous mandible of each of 5 female minipigs, by applying different drilling protocols (standard [SD, control] *vs.* oversized [OD, test]). SD included a 3-step series of drills without profiling of the cortical bone (IDD = 0.3 mm). OD included a 5-step series of drills and profiling of the cortical bone which resulted, respectively, in two IDD along the implant axis: (1) IDD = 0.1 mm in the 8-mm central apical length of the osteotomy; and (2) IDD = 0.0 mm in the 3.5-mm coronal length. Maximum ITV was recorded and non-submerged healing was allowed for 12 weeks ($n = 5$). Oversized surgical preparation of the implant site yielded low ITV at tapered implants with a progressive thread geometry, but enhanced osseointegration and peri-implant bone density, and preserved better the MBL.

LIST OF PAPERS

This dissertation is based on the following five papers, which will be referred to in the main text by their Roman numerals. The papers are appended at the end of the thesis.

- I. Histomorphometric evaluation of a dual acid-etched vs. a chemically modified hydrophilic dual acid-etched implant surface. An experimental study in dogs.
de Jesus RNR, Stavropoulos A, Oliveira MTF, Soares PBF, Moura CCG, Zanetta-Barbosa D.
Clinical Oral Implants Research. 2017,28(5): 551-557.
- II. Interfacial biomechanical properties of a dual acid-etched versus a chemically modified hydrophilic dual acid-etched implant surface: an experimental study in Beagles.
de Jesus RNR, Carrilho E, Antunes PV, Ramalho A, Moura CCG, Stavropoulos A, Zanetta-Barbosa D.
International Journal of Implant Dentistry. 2018,4(1): 1-10.ic
- III. Differential modulation of osteoclastogenesis by macrogeometrically designed hydrophobic and chemically modified hydrophilic titanium surfaces.
de Jesus RNR, Tsatsanis C, Moura CCG, Zanetta-Barbosa D, Stavropoulos A.
(Submitted)

- IV. Oversized surgical preparation of the implant site: systematic review and meta-analysis of preclinical studies.
de Jesus RNR, Pandis N, Zanetta-Barbosa D, Stavropoulos A.
(In manuscript)
- V. Effect of oversized implant site preparation on osseointegration of submerged tapered titanium implants: a study in the minipig mandible.
de Jesus RNR, Papadimitriou S, Zanetta-Barbosa D, Stavropoulos A.
(In manuscript)

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Contribution by the respondent

The respondent performed most of the work from planning, experimental work (with exception of the histotechnical procedures), to data analysis. The respondent was also the main contributor to writing the manuscripts.

ABBREVIATIONS AND ACRONYMS

Abbreviations

α	Alpha
β	Beta
θ	Contact angle
$^{\circ}\text{C}$	Degree Celsius
$\varnothing$	Diameter
κ	Kappa
%	Percentage
2D	Two-dimensional
3D	Three-dimensional
ANOVA	One-way analysis of variance
AOI	Area of interest
APCs	Antigen presenting cells
ARRIVE	Animal Research: Reporting of In Vivo Experiments
B cells	Lymphocytes/humoral specific immune response
BD (%)	Peri-implant bone density percentage
BIC (%)	Bone-to-implant contact percentage
BL	Bone level
BLCs	Bone lining cells (flattened inactive osteoblasts)

BMMs	Bone-marrow-derived macrophages
BMSCs	Bone-marrow-derived stems cells
BMUs	Basic multicellular units
BSA	Bovine serum albumin
BTE	Bone tissue engineering
Ca²⁺	Calcium ions
CaCO₃	Calcium carbonate
CaF₂	Calcium fluoride
Ca₁₀(PO₄)₆(OH)₂	Hydroxyapatite crystals
Ca₃(PO₄)₂	Calcium phosphate
CBL	Peri-implant crestal bone loss
CCPC	Cells seeded directly on polystyrene-surface wells
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CEUA	Bioethics Committee for Animal Experimentation
CLSM	Confocal laser scanning microscopy
cm	Centimeter
CO₂	Carbon dioxide
CONCEA	National Council for Animal Control and Experimentation
Cp	Commercially pure
CRI	Constant rate infusion
C_t	Cycle values
DAMPs	Damage-associated molecular patterns
DAPI	4',6-diamidino-2-phenylindole
DCs	Dendritic cells
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid

DNase	Deoxyribonuclease
(d)NTPs	(deoxy)nucleotide triphosphates
ECM	Extracellular matrix
EH	External hexagon connection system
ERT	Endogenous regenerative technology
F⁻	Fluoride
F-actin	Actin filaments
FAM	6-carboxyfluorescein dye
f-BIC (mm)	First BIC, millimeter
FBR	Foreign body reaction
FBS	Fetal bovine serum
FELASA	Federation for Laboratory Animal Science Associations
FSD	Functional secretory domain
FTE	Fibrous tissue encapsulation
g	Gram
GBR	Guided bone regeneration
GM	Grand Morse [®] implant-abutment connection interface
HA	Hydroxyapatite
h-BIC (%)	Host BIC percentage
HCl	Hydrochloric acid
HLX	Helix [®] , tapered implants with a progressive thread design
H₂SO₄	Sulfuric acid
I²	Test for homogeneity
IDD	Implant-last drill discrepancy
IGF	Insulin-like growth factor
i.m.	Intramuscular

IQR	Interquartile range
ISO	International Organization for Standardization
ISQ (%)	Implant stability quotient percentage
ITV (N.cm)	Insertion torque value, Newton centimeter
IU	International Units
J	Joule
kg	Kilogram
kpa	Kilopascal
L	Length
LB	Lamellar bone
LPS	Lipopolysaccharides
Mϕ	Macrophage
Mϕ1	Classically activated/pro-inflammatory macrophage phenotype
Mϕ2	Wound healing/anti-inflammatory macrophage phenotype
M	Machined
MBL	Marginal peri-implant bone level
MeSH	Medicinal subject headings
mg	Milligram
MGB	Minor groove binder
MgCl₂	Magnesium chloride
MIQE	Minimum Information for Publication of qPCR
ml	Milliliter
mm	Millimeter
MNGCs	Multinucleated giant cells
Mo	Monocytes
MPa	Megapascal
m-RNA	Messenger ribonucleic acid
MSCs	Mesenchymal stem cells

MSFA	Maxillary sinus floor augmentation
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5 Diphenyltetrazolium Bromide
N	Newton
NA	Numerical aperture
NaCl	Sodium chloride
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
ng	Nanogram
NK	Natural killers
NO	Nitric oxide
NSD	No significant difference
OBs	Osteoblast
OCs	Osteoclast
OCN	Osteocalcin
OD	Oversized drilling
OHRQoL	Oral-health related quality of life
OLCS	Osteocytic lacunar-canalicular system
Oligo(dT)	Oligonucleotide deoxythymine
PA	Polyamides
PAMPs	Pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PICO	Population, intervention, comparison, outcome
PGA	Polyglycolic acid
pH	Potential hydrogen
PLA	Poly-L-lactic acid
PMNs	Polymorphonuclear leucocytes
PO³⁻₄	Phosphate ions

Poly(A) tail	Polynucleotide chain consisting of adenosine monophosphates
PRISMA	Preferred Reporting of Systematic Review and Meta-analysis
PTH	Parathyroid hormone
qPCR or RT-qPCR	Reverse transcriptase real time (quantitative) polymerase chain reaction
r	The Spearman's correlation coefficient
R_a (μm)	Average height amplitude (2D parameter)
RA	Resorption apparatus
rad	Radian
RAW 264.7	Immortalized, murine macrophage-like cell line
RAW-OC	Macrophages treated for differentiation to osteoclasts
RB	Ruffled border
RER	Rough endoplasmic reticulum
Rho	Rank-Order
RNases	Ribonucleases
RNA	Ribonucleic acid
RoB	Risk of bias
rpm	Rotations per minute
r-RNA	Ribosomal RNA
RT	Reverse transcription
RTQ	Removal torque test
RTV (N.cm)	Removal torque value
S_a (μm)	Average height amplitude (3D)
SAE	Sandblasted, dual acid-etched
SAE-HD	Sandblasted, dual acid-etched surface with ad- ditional chemical treatment
SD	Standard drilling

s.d.	Standard deviation
S_{dr} (%)	Mean slope and the developed area ratio percentage
S_{ds} (1/mm²)	Number of peaks per area unit
SEM	Scanning electron microscopy
SLA	Sandblasted, large grit, acid-etched surface
SLActive/ modSLA	Chemically modified sandblasted, large grit, acid-etched surface
SM	Schneiderian membrane
S_{sk}	Amplitude distribution curve
SST	Stainless steel
SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
S_z (μm)	Average distance between the highest peak and the deepest valley
SZ	Sealing zone
TaqMan	Target-specific, dual-labeled hydrolysis (oligo) probe
T cells	Lymphocytes
Th	Cytotoxic lymphocytes/cell-mediated specific immune response
TiCl₄	Titanium tetrachloride
TiO₂	Titanium oxide
Ti-Zr	Titanium-zirconium alloy
TLRs	Toll-like receptors
Tm	Primer melting temperature
TMX	Titamax® CM EX, cylindrical implants with double triangular threads
Treg	Regulatory lymphocytes

t-RNA	Transfer RNA
μg	Microgram
μl	Microliter
μm	Micrometer
μM	Micromolar
UN	Undersized drilling
$\mu\epsilon$	Microstrain
UV	Ultraviolet
VAM	Value of actual micromotion
χ^2	Chi-square test
XPS	X-ray photoelectron spectroscopy

Chemical element symbols

Al	Aluminum
C	Carbon
Ca	Calcium
Cl	Chlorine
Co	Cobalt
Cr	Chromium
F	Fluorine
Fe	Iron
H	Hydrogen
Mg	Magnesium
N	Nitrogen
Na	Sodium
O	Oxygen
P	Phosphorus
S	Sulfur
Sr	Strontium

Ti	Titanium
V	Vanadium
Zr	Zirconium

Gene and protein symbols

$\alpha_v\beta_3$	Integrin (vibronectin receptor)
1α,25-(OH)$_2$D$_3$	Calcitriol
ALP	Alkaline phosphatase
AP	Acid phosphatase
AP-1	Activator protein 1
BMP	Bone morphogenetic protein
BSP	Bone sialoprotein
C	Complement system
C3b	Complement component 3b
C5a	Complement component 5a
CA2	Carbonic anhydrase II
CALC	Calcitonin
CALCR	Calcitonin receptor
CBFA-1	Core-binding factors subunit alpha-1
COL	Collagen
CTGF	Connective tissue growth factor
CTSK	Cathepsin-K
FGF	Fibroblast growth factor
Fn	Fibronectin
IκK	Inhibitor of κ B kinase complex
IL-1β	Interleukin 1 beta
IL-4	Interleukin 4
IL-6	Interleukin 6

IL-8	Interleukin 8
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-17	Interleukin 17
MAPKs	Mitogen-activated protein kinases
M-CSF	Macrophage colony-stimulating factor
MITF	Microphthalmia-associated transcription factor
MMP-9	Matrix metalloproteinase 9
OPG	Osteoprotegerin
OPN	Osteopontin
PAF	Platelet activating factor
PDGF	Platelet derived growth factor
RANK	Receptor activator of nuclear factor kappa B
RANKL	RANK ligand
Runx2	Runt-related transcription factor 2 gene
Smads	Transducers for membrane receptors of TGF- β
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor alpha
TRAF	TNF receptor-associated factor
TRAP1	Tartrate-Resistant Acid Phosphatase (type 5b AP)
VEGF	Vascular endothelial growth factor
Vn	Vibronectin
WNT	Wingless-related integration site

INTRODUCTION

*I seem to have been only like a boy playing on the seashore,
and diverting myself in now and then finding a smoother pebble
or a prettier shell than ordinary, whilst the great ocean
of truth lay all undiscovered before me*

Sir Isaac Newton (1643 - 1727), English physicist

Partial and complete edentulism (*i.e.*, tooth loss; Figure 1a) is a major chronic condition caused by multifactorial reasons (*e.g.*, periodontal disease [69.20%], endodontic treatment failure [25.67%] and trauma [6.20%]), described as the “the final burden for oral health”^{1,2}. It is related to the socio-economic development in different demographic populations worldwide, mostly in low- and middle-income countries. The global prevalence of complete edentulism has remained stable throughout the last 3 decades (approximately 10% of the population), despite an increase in average life expectancy³. Nevertheless, this condition has a series of deleterious consequences for general and oral health-related quality of life (OHRQoL), which deeply affect emotional well-being and increase mortality rates⁴.

This condition is regarded as a contributing factor in disease progression of numerous systemic conditions due to malnutrition pathogenesis. Nutritional deficiency is primarily caused by chewing disability and, consequently, restricted ingestion of functional nutrients. Consequently, patients reveal high intake of saturated fats, which increases cholesterol levels and risk of obesity⁵, cardiovascular diseases (*e.g.*, hypertension, aortic valve sclerosis, and coronary heart disease)^{6,7}, gastrointestinal disorders and pancreatic cancer^{8,9}, diabetes type II¹⁰, chronic kidney disease¹¹, and sleep-disordered breathing (*e.g.*, obstructive sleep apnea)¹².

Figure 1. Clinical case of a 60-year-old male patient. (a) A pre-operative intra-oral photo presenting complete edentulism. (b-d) One-year post-operative intra-oral photos after rehabilitation with a maxillary complete denture and a mandibular implant-supported overdenture. (e) One-year post-operative radiographic image showing the three osseointegrated implants at symphysis and parasymphysis region. Image courtesy of Prof. Dr. Darceny Zanetta-Barbosa, P-I Brånemark Institute, Bauru, SP, Brazil.

Moreover, edentulism leads to several orofacial esthetic and functional complications (*e.g.*, unfavorable intermaxillary relationship). Traditionally, removable denture and tooth-supported fixed prosthesis comprised distinctive procedures in the treatment of totally

and partially edentulous patients, respectively, demonstrating high patient satisfaction¹³. However, the most common lesions affecting the oral mucosa in these patients include denture stomatitis (inflammatory condition of the palatal mucosa caused by complete denture wearers), oral candidiasis, oral dyskinesia (involuntary orofacial movements), and traumatic ulcers, apart from a loss of retention¹⁴. Altogether, these injuries increase individual exposure to external and internal pathogens and the risk of malignancy^{15,16}.

Over the past few decades, dental materials have undergone extensive development concomitantly with the establishment of implant dentistry as a field of science in the 1980's¹⁷. Oral implants are endosseous fixtures engineered to osseointegrate into the maxillofacial bone to functionally support dental prostheses in replacing missing teeth (implant-supported prostheses), similar to a natural tooth root and crown (Figure 1b-e). Implant rehabilitation of edentulous patients has become a predictable routine treatment in various modalities: single and multiple implants supporting fixed or bar-retained prostheses (*i.e.*, overdentures) in anterior and posterior regions of the maxilla and mandible. High long-term survival rates (90-95%) – whether an implant remains in situ irrespective of clinical alterations during the observation period¹⁸ – are reported for the major dental implant systems after 5-10 years in function in a variety of clinical situations¹⁹⁻²³.

Implant stability is a valuable clinical measurement of the interfacial strength against micromotion upon surgical insertion (mechanical/primary stability) and in the post-osseointegration period (biological/secondary stability), which is considered a prerequisite for successful osseointegration²⁴. In context, Albrektsson et al. (1981)²⁵ reported six factors that may influence primary stability: material biocompatibility, surface technology (*e.g.*, micro- to nanotopography and chemical composition), implant macrogeometry (*e.g.*, implant body and thread design), state of the host site (*e.g.*, recipient bone type and volume), surgical preparation of the implant site (*e.g.*, osteotomy procedure), and loading conditions. Despite high long-term survival rates, implant failure occurs in connection with multifactorial conditions associated with the abovementioned aspects as well as the patient's systemic health²⁶

(see the section “prognostic factors and long-term maintenance of the peri-implant bone support”).

A large and growing body of literature has investigated the effects of various surface modifications to improve the quality and rate of osseointegration necessary to prevent aseptic loosening and to allow time-critical functional loading (so-called immediate or early loading). A number of studies have consistently shown that surface properties positively modulate the early period of wound healing. However, a major problem lies on the fact that most of the recent research neglects the hierarchical effects of implant macrodesign and surgical preparation of the implant site, defined herein as implant hardware design, on osseointegration relative to the status of the host bone. In this regard, this thesis is focused on aspects of technological development of Titanium (Ti) implants and osseointegration for application in oral implantology.

Titanium as a biomaterial

Biomaterials refer to bioengineered devices and substances, natural and synthetic, intended to interact with biological systems towards a medical purpose with either diagnostic or therapeutic applications to treat, augment, repair, and reconstruct lost, damaged, and diseased tissues²⁷. Biomaterials science is a multidisciplinary subject encompassing chemical, mechanical and electrical engineering, biology, dentistry, materials science, among others²⁸. Throughout the last few decades, major technological and innovative developments in this field resulted in a vast range of biological and synthetic biomaterials mainly incorporating metals, ceramics, and polymers, but also nanoparticles²⁹.

Overall, they cover three classes of biocompatible materials (*i.e.*, materials that do not stimulate adverse immune reactions): bioinert, bioresorbable and bioactive²⁸. Bioinert materials are foreign bodies known to trigger fibrous encapsulation due to its toxicity (*e.g.*, stainless steel [SST], cobalt-chromium [Co-Cr] alloy, titanium [Ti], zirconium [Zr], aluminum [Al] and polyamides [PA]. Bioresorbable materials dissolve in contact with fluids (*e.g.*, magnesium alloys [Mg], polyglycolic acid [PGA] and poly-L-lactic acid [PLA])³⁰. Finally, bioactive materials refer to components capable of stimulating a positive biological response in the living tissue,

such as activation of specific genes related to the regulation of bone formation (*e.g.*, bioglass, hydroxyapatite, proteins, ions, polysaccharides, and drugs)^{31–36}.

Titanium (origin: *Titans* from Greek mythology) is a low-density metal (4.5 g/cm³) with the highest worldwide application in biomedical engineering, particularly in oral implantology, cranio-, maxillofacial and oral surgery, and orthopedics due to its outstanding properties that make them suitable for medical applications³⁷.

Even though titanium is a transition and reactive element, its high biocompatibility is attributable to the low toxicity and low tendency to release ions (isotypes) from its surface. These properties are further recognized to be a result of an inert surface oxide film spontaneously formed upon exposure to air and aqueous electrolytes, with a thickness of 2–5 nm. This dense and stable titanium dioxide layer (TiO₂) provides high resistance to corrosion (*i.e.*, process of gradual damage by salt solutions and organic acids)³⁸.

Commercially pure titanium (CpTi) is the gold standard material for the fabrication of oral implants with high biomechanical properties (*e.g.*, tensile strength and strength-to-weight ratio), which is defined by its chemical composition³⁹. The latter consists of, approximately, 99.7% titanium while the remaining 0.3% contains a mix of different elements, including oxygen (O₂), iron (Fe) and carbon (C). Titanium is available in four grades exhibiting minor differences in the concentration of O₂ that substantially alters its physical and mechanical properties.

In spite of the fact that titanium was already employed as a bone-anchoring material in the middle of the 20th century, the advent of its application in dentistry began in the 1960s alongside the unforeseen discoveries made by the Swedish orthopedic surgeon Per-Ingvar Brånemark, who investigated marrow microcirculation employing CpTi grade I^{40–42} (see the section “osseointegration: on the evolution of modern implant dentistry”).

Currently, CpTi grade IV is the most extensively applied material in implant dentistry. Its tensile strength is around 560 MPa given the high concentration of Fe, in comparison with 360 MPa of CpTi grade II. The commercial manufacture of this metal is by the metal-thermic reduction of Ti tetrachloride (TiCl₄) under a Ni-protective atmosphere⁴³. At room temperature, CpTi has a hexag-

onal close-packed (hcp) crystal matrix, called α -phase. In turn, when heated up to 883°C, CpTi suffers an allotropic phase transformation through which a bcc matrix is formed, termed β -phase.

Accordingly, CpTi can be alloyed with other elements to improve its physical properties to allow clinical applications where higher strength is required to withstand functional loading (*e.g.*, smaller diameter implants, parafunctional occlusion, etc.) while maintaining similar biocompatibility. Alteration of the α/β -phase improves the properties of Ti and its alloys for an extensive range of applications, giving the optimum quality of mechanical and corrosion resistance⁴⁴.

For instance, the most commonly used alloys in both dental and orthopedic fields consist of titanium, aluminum, and vanadium (Ti-6Al-4V; grade V) with a tensile strength at around 940 MPa. Although V stabilizes the α/β -phase, the corrosion resistance of this alloy is inferior to that of CpTi⁴⁵. When modifying the surface properties of Ti-6Al-4V in concentrated sulfuric acid, for example, the TiO₂ layer is slowly dissolved, unleashing unstable elements (Al and V). Potential biological complications, such as unsolving inflammatory response and systemic toxicity, may result from adverse effects of corrosion and particles released in the surrounding tissues⁴⁶.

Recently, a binary alloy (titanium-zirconium alloy; Ti-Zr) was introduced in the market as an alternative implant material, supposedly presenting similar mechanical resistance compared to Ti-6Al-4V, and thereby suited for producing narrow diameter implants. However, Anchietà et al. (2014)⁴⁷ validated the nanomechanical stability of Ti-Zr alloy implants (13-17% Zr) compared to CpTi implants, showing similar low elastic modulus (a measure of the material stiffness – the stiffer the material, the greater the modulus and the smaller the strain) and hardness (measure of the material resistance to strain) in an *in vivo* model of minipig mandible.

Osseointegration: on the evolution of modern implant dentistry

The earliest evidence of osseointegration, only recently reported in the literature, is probably an ancient anthropological sample of a

dental root-shaped iron implant ankylosed to the posterior maxillary bone of a skeleton dated to the first or second century AD⁴⁸.

Early attempts to insert subperiosteal bioinert fixtures, the so-called first generation of implants, did not succeed due to lack of biocompatibility and excessive metal corrosion, thereafter causing adverse clinical response associated with extensive bone resorption^{49,50}. Successive reports on high failure rates of stainless-steel implants inserted into the jawbone of total edentulous patients portrayed the second generation of implants characterized by fibrous encapsulation (*i.e.*, formation of a connective tissue fiber network as a result of foreign body reaction [FBR])^{51,52}.

The development of modern implant dentistry began almost half a century ago and stands on the groundbreaking discoveries made by Per-Ingvar Brånemark. Following experiments conducted in a model of rabbit femur to study blood flow, Brånemark reported that the bone completely integrated into threaded implant chambers. This component could not be removed without, otherwise, fracturing the bone^{41,53}. His finding was later termed as osseointegration in 1977. From the Greek *osteon*, meaning bone, and the Latin *integrare*, which means to make whole, osseointegration was originally defined with respect to histological criteria as “the direct contact between foreign materials and bone without any interposed soft tissue layers”⁴⁰.

In the middle of the 1960s, Brånemark et al. gradually initiated preclinical experiments in long bones of dogs, and then clinical trials evidencing the first successful integration of a titanium implant into the jawbone of a man⁵⁴. Observations at the light microscopic level validated a better distinction of direct bone-to-implant contact (BIC) between successfully osseointegrated implants and those with fibrous encapsulation⁵⁵.

In the 1980s, a second definition was given to osseointegration as a “direct structural and functional connection between ordered, living bone and the surface a load-carrying implant”⁵⁶. Fascinatingly, this paradigm has guided each scientific theory proposed since then, proclaiming implant dentistry as a field of science.

After numerous experimental trials in human subjects to confirm this unique phenomenon, a more clinically oriented definition was formulated, in 1991, as “a process in which clinically asymptomat-

ic rigid fixation of alloplastic materials is achieved and maintained in bone during functional loading”⁵⁷.

Most recently, the role of Ti implants to activate and modulate the immunoinflammatory response during wound healing has been of major interest. Hence, the most recent suggested definition of osseointegration refers to the alleged observations on the normal reaction of bone to sequester any implanted foreign body. This theory states that “osseointegration is a foreign body reaction where the interfacial bone is formed as a defense reaction to shield off the implant from the tissues”⁵⁸. Evidently, the phenomenon of osseointegration is yet a matter of debate despite extensive research following the years after its breakthrough.

A dental implant is appropriately designed to offer mechanical resistance to support normal prosthetic functional loading. The conventional protocol proposed by Brånemark for treatment with dental implants (Brånemark System) consisted of two phases^{17,40}. Primarily, the instrumentation of the residual alveolar bone and implant installation comprised the “surgical phase”. In the second place, the “prosthetic phase” included the preparation and installation of the prosthesis to guarantee functional loading and restore normal chewing ability. A stress-free submerged healing period of 3 and 6 months following the surgical phase was recommended for mandibular and maxillary implants, respectively, to allow successful osseointegration prior to prosthetic installation.

This protocol supported the installation of 4 implants in the rehabilitation of total mandibular edentulism, whereas 6 implant fixtures were indicated to be inserted in the maxilla with minimal to moderate resorption. The exact period to allow complete integration of implants was not scientifically grounded at that time but only regarded as a clinical interpretation. Despite this, the original protocol yielded predictable success rates, providing a foundation to current and future evolution of treatment modalities to all types of edentulism^{59,60}.

Over the years, profound modifications in surgical protocols resulted in a paradigm shift in the treatment of edentulous patients with the advent of immediate functional implant loading, firstly reported in 1990⁶¹. Immediate loading is provided within the first 24 – 48 h after surgical implant placement, whereas early loading

comprises prosthetic rehabilitation of implants within 14 days post-operatively⁶².

For instance, immediately loaded Brånemark implants presented a 10-year survival rate of 84.7% compared to 100% for submerged implants placed into mandibular sites⁶³. However, a recent study has shown that immediate functional loading of implants in the posterior mandible, in addition to improving implant stability and promoting minimal peri-implant crestal bone loss (CBL < 1 mm), does not affect their long-term prognosis with a survival rate of 100%⁶⁴. Regarding implant-supported overdentures (anterior mandible), immediately loaded prostheses show comparable long-term success and survival rates (91.6% and 97.6%, respectively) compared to delayed loading⁶⁵. In the aesthetic zone of the maxilla, immediately restored single implants showed a short- and long-term success rate of 92.9% and 100%, respectively⁶⁶. Comparably, in the posterior maxilla, immediate loading of single implants reveals lower short-term survival rate (92.8%)⁶⁷ when compared to multiple implants supporting fixed (98.6%)⁶⁸ or bar-retained prostheses (97.1%)⁶⁹.

MECHANISMS OF HOST-TO-IMPLANT INTERACTIONS

Bone architecture and structural composition

Bone is a complex living tissue presenting a profoundly specialized, metabolically active, mineralized extracellular matrix (ECM) with a complex multiscale composition and macroscopic geometry under continuous remodeling across the human life span⁷⁰. This tissue exerts vital primary functions, including structural support, calcium (Ca) metabolism (acid-base homeostasis and pH regulation), harboring of bone marrow, and lodging of hematopoiesis (formation of cellular components of blood)⁷¹. The numerous constituents of bone confer remarkably resistant and adaptive mechanical properties while preserving its elasticity⁷².

A distinct macroscopic characteristic of all types of bone is a dense outer sheet of cortical (compact bone) and a central, multifaceted spatial system occupied by bone marrow, which is interrupted by bone trabeculae (cancellous or spongy bone)⁷³. Compact bone consists of a solid continuous mass deprived of macroscopically visually spaces. Cancellous bone encompasses a three-dimensional (3D) framework of bony spicules or trabeculae delineating the medullary cavity, particularly at the extremities of long bones. Both cortical and trabecular bone are integrated into one single structure without a visible margin and present different mineral density, porosity, 3D structure, and metabolic responses with opposing behavior to varied stimuli^{74,75}.

Cortical bone, presenting a porosity of 5 to 10%, provides maximum resistance to deformation and bending forces (*i.e.*, compressive strength). Cancellous bone presents a greater surface area per

unit volume with a porosity of 50 to 90%, intended to maximize nutrient diffusion and exposure to circulating cytokines and hormones vital in mineral homeostasis. Consequently, it possesses a higher ratio of metabolic and remodeling activity due to the presence of bone progenitor cells in closer proximity to circulating molecules⁷⁶.

More than three decades ago, Lekholm and Zarb (1985)⁷⁷ proposed a bone classification for residual edentulous arches based on bone resorption pattern and bone quality/type in terms of cortical to trabecular bone ratio. The first classification considered the severity of horizontal and vertical dimensional changes of the edentulous alveolar ridge, from type A, characterizing a well-preserved bone volume, to type E, when there is extreme resorption of basal bone – the latter supports the alveolar process.

According to the amount of bone mass per unit volume in different regions of the jaw, bone quality is classified into four qualities/types⁷⁸ (Figure 2):

- Quality 1: almost the entire bone is composed of homogeneous compact bone (anterior mandible).
- Quality 2: a thick layer of compact bone surrounds a core of dense trabecular bone (posterior mandible).
- Quality 3: a thin cortical bone surrounds a dense trabecular bone of favorable strength (anterior maxilla).
- Quality 4: a thin layer of cortical bone surrounds a core of low-density trabecular bone of poor strength (posterior maxilla).

Figure 2. A graphic illustration of the Lekholm & Zarb classification based on bone quality/type in terms of cortical to trabecular bone ratio in different regions of the edentulous jaw. This image was adapted from Alghamdi et al. (2018)⁷⁸ it is licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0).

Most of the outer aspect of every compact bone is surrounded by periosteum, consisting of two layers of specialized connective tissue (an inner osteogenic layer populated by osteoprogenitor cells and an outer fibrous layer) in the presence of a rich microvascular supply⁷². The internal surfaces of the marrow cavity of cortical and cancellous bone are covered by endosteum, a thin loose connective tissue containing osteogenic cells that also possesses osteogenic properties, however in a minor degree.

Irrespective of the type of structural composition, mature bones consist of a mineralized interstitial substance deposited in microscopic layers of lamellae (3 to 7 μm), named bone matrix⁷⁹. There are three distinct types of layering:

- Circumferential lamellae: enclose the entire adult bone, forming its outer and inner boundaries.
- Concentric lamellae: constitute the bulk of compact bone and form its basic metabolic unit, the osteon (*i.e.*, Haversian system).
- Interstitial lamellae: fragments of pre-existing concentric lamellae created as a result of bone remodeling.

The bone microstructure is organized in different levels of hierarchy. Osteon is the main functional component of cortical bone, oriented to its long axis (Figure 3). A canal is located in the center of each osteon, the Haversian canal, harboring a capillary. Adjacent Haversian canals are interconnected by Volkmann canals, channels that contain blood vessels and create a vascular network throughout compact bone.

At the microscopic level, bone is classified into four types. First and foremost, woven bone (WB, primary or phase I bone; Figure 4a) plays a principal role in healing because of its high formation speed (≈ 30 to $60 \mu\text{m/day}$), high vascularity, soft tissue content, and bone cellularity⁸⁰. As a result, it is formed in a very disorganized fashion deprived of lamellar architecture or Haversian system with limited biomechanical strength. During healing, WB is resorbed and replaced by mature lamellar bone (LB, secondary or phase II bone; Figure 4b). Composite bone refers to the transitional state between phase I and II bone in which a WB lattice filled with LB is observed. The latter is the most abundant, mature, load-

bearing bone in the body. This type of bone forms slowly (approximately 0.6 to 1 $\mu\text{m}/\text{day}$) and contains a well-organized mineralized structure consisting of multiple lamellae.

Figure 3. A graphic illustration and representative micrograph of compact bone microstructure. (a) A cross-sectional view showing the basic metabolic unit, osteon, formed by layers of concentric lamellae and the Haversian canal in the center harboring blood vessels, nerve, and lymphatic vessel, interconnected by Volkmann canals. **(b)** A micrograph of an osteon demonstrating the concentric and circumferential lamellae. Magnification at x40. These images were created by Rice University ©1999-2016 and they are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0). Download for free at <http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@8.24>.

Figure 4. Optical micrographs showing the microstructure of WB and LB around and in direct contact to the implant surface (in black). (a) Trabeculae of WB occupying the central portion of the chamber after two weeks of healing. **(b)** At twelve weeks of healing, mature bone and areas of bone marrow are in direct contact with the implant surface. LB, lamellar bone. WB, woven bone. Original magnification at x100. These images were adapted from Abrahamsson et al. (2004)¹⁷¹, ©John Wiley and Sons, Inc (license number: 4519360471641).

The sub-microscopic bone matrix complex system is formed by two major components: an organic matrix and inorganic salts. At the molecular level, the organic composition of bone is comprised of, approximately, 95% of collagen (COL; primarily type I, but also contains small amounts of COL-V), exhibiting a high degree of intermolecular bonding. COL fibers are three-dimensionally organized in a cross-linked matrix in mature lamellae, offering maximal bone strength and elasticity. The inorganic component of bone consists mainly of hydroxyapatite crystals ($\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$; *i.e.*, apatite) nucleated into the collagenous fibers. It contains calcium phosphate ($\text{Ca}_3[\text{PO}_4]_2$; 85%), calcium carbonate (CaCO_3 , 10%), calcium fluoride and magnesium fluoride (CaF_2 ; 5%), as well as small amounts of non-collagenous proteins and peptides (*e.g.*, phospholipids and proteoglycans). These mineral salts, which are regulated by bone homeostasis, affect the solubility of bone mineral and, consequently, its stiffness and compressive strength. The superior stiffness of bone is, therefore, defined by its inorganic

components, while its elasticity is attributed to its organic matrix^{81,82}.

Bone mechanical properties

Three types of forces may be applied to the bone during implant placement, under passive intraoral forces (*i.e.*, exerted by the tongue and musculature) and functional loading: compressive and shear stress, and tensile strength⁸³. As an anisotropic tissue, the mechanical stimulus will cause bone behavior in the stress-strain domain (*i.e.*, bone deformation relative to the implant length) according to its direction, magnitude, rate, and duration⁸⁴. Ultimately, changes in the bone morphology, microstructure, and bulk density occur as a result of biomechanical adaptation.

As previously mentioned, the bone volume, density, and cortical thickness of the mandible and maxilla vary according to the anteroposterior region, as so does bone strain. Mandibular cortical thickness varies significantly between sites, ranging from 3.7 (s.d. = 0.9) in the anterior region to 1.4 mm (s.d. = 0.1) posteriorly⁸⁵. On the other hand, maxillary cortical thickness varies from 1.0 to 2.1 with the thinnest site in the anterior region⁸⁶.

With regard to mineral density, trabecular bone reveals an average value of 0.85 - 1.53 g/cm³ in the mandible⁸⁷, while cortical bone reveals higher values (1.85 - 2.0 g/cm³)⁸⁵. In turn, the average bone density in the maxilla is 0.67 g/cm³, considering both compact and cancellous bone⁸⁸ with, however, greater anteroposterior variability⁸⁹. Certainly, mean values are representative of the adult population (> 50 years old) and may change with age, gender, ethnicity, and systemic conditions.

Similarly, elastic modulus differs between mandible (18.3 GPa) and maxilla (14.9 GPa)⁸⁸, revealing a strong positive correlation with bone density. The posterior aspects of the jawbones present significantly higher elastic modulus (17.5 GPa) than the anterior aspects (15.7 GPa), possibly due to higher biomechanical adaptation to stress. Thus, bone quality and density of the trabeculae, characterized by a high ratio of metabolic activity and low elastic modulus, is one of the main determinants of absorption and dissipation of forces⁹⁰, where the highest concentration is detected at the implant apex⁹¹.

Considering loading forces, Julius Wolff, more than one century ago, postulated on the stimulating potential of mechanical stimulus to support bone remodeling in the microarchitecture of the trabeculae and, subsequently, changes in the cortical dimension⁹². According to Wolff's law, in the presence of physiological dynamic strain (*i.e.*, cyclic change in internal strain due to functional loading), bone apposition is accelerated resulting in thicker and more resistant bone tissue, whilst areas found in absence thereof or exposed to overloading are likely to undergo bone resorption.

This theory has been extensively investigated in the field of implant dentistry⁹³. There is an indication that organization of cortical bone microstructures (*e.g.*, osteon diameter and morphology owing to collagen fibers orientation and lamellar arrangement) is associated with the level of stress distribution and strain mode, magnitude, and energy⁹⁴. All these parameters are directly related to the level of frictional force and compressive stress generated at the bone-implant interface. For instance, bone compression results in transverse osteonic structures and more rigid bone, while tensional strength creates longitudinal osteons⁹⁵.

Cellular components of bone tissue

Bone marrow is populated by cells of the immune system that regulate inflammation and the host defense systems, such as dendritic cells (DCs), granulocytes/polymorphonuclear leucocytes (PMNs; *e.g.*, neutrophils), monocytes (Mo), macrophages (M ϕ), B lymphocytes, and T lymphocytes (regulatory [Treg], T helper [Th], and cytotoxic [T] cells)^{96,97}. In actively growing bones, two main cell lineages are responsible for the skeletal development, bone metabolism, and physiology: (1) osteogenic cells, chiefly responsible for bone formation and maintenance; and (2) osteoclasts (OCs), bone-resorbing cells⁹⁸.

Osteogenic lineage

Pluripotent stem cells of mesenchymal origin (MSCs) are located on or near all the free surfaces of developing or growing bones. They are capable of self-renew and generate numerous differentiated progenitor cells, including commitment and differentiation into mature osteoblasts (OBs) in response to multiple stimuli, fractures

and other forms of injury⁹⁹. Particularly, osteogenic cells have a variable morphology characterizing different maturational and functional stages: osteoprogenitor cells, preosteoblasts, osteoblasts, osteocytes, and bone lining cells (BLCs)¹⁰⁰.

OBs are mononucleated cells that synthesize collagenous and non-collagenous bone matrix proteins to produce the organic matrix of bone (osteoid), which acts scaffolding for the deposition of apatite. Mature OBs are arranged in an epithelioid layer of either cuboidal cells during deposition of bone matrix or slightly flattened cells connected to one another in an active growth area, frequently referred to as endosteal or periosteal OBs. They form a continuous form a cell layer over the forming bone surface, which is assumed to act as a protective barrier, controlling the flux of ions.

As osteoblasts deposit bone, they become embedded in the matrix and decrease in size, then called osteocytes¹⁰¹. Other OBs undergo apoptosis or experience certain cytological integration to an earlier developmental stage (dedifferentiation), clustered in the form of shelter (BLCs) while keeping their osteogenic potential through reactivation of secretory domains^{102,103}.

Osteocytes are the most abundant bone units, allowing cell-to-cell communication throughout narrow dendritic processes enclosed in canaliculi that emerge from the lacunae¹⁰⁴. During active bone deposition, such prolongations create an uninterrupted network of contiguous gap-junctional processes with the adjacent osteocyte lacunae, BLCs and the surrounding tissue (*i.e.*, osteocytic lacunar-canalicular system [OLCS]).

Osteocyte cell function and survival are entirely dependent on the diffusion of oxygen, nutrients, and waste via the OLCS¹⁰⁵. Furthermore, this complex system is responsible for mechanotransduction – a physiological process by which physical entities (*e.g.*, topographical and substrate rigidity) and biomechanical stimuli (*e.g.*, stress and hydrostatic pressure) are converted into biochemical signals throughout multicellular mechanisms and signaling systems¹⁰⁶ – responsible for homeostatic regulation and tissue repair upon prospective deleterious stress and/or injury^{107,108}. This process is possible due to receptor-mediated proteins such as integrins, cadherins, ion channels, and cilia that stimulate cell-to-cell communication^{109,110}.

Osteoclasts

Multinucleated bone-resorbing cells, osteoclasts originate from the hematopoietic bone-marrow-derived myeloid lineage precursors (Mo and M ϕ) and are activated both in basal and pathologic conditions, exerting a central role in the regulation of bone remodeling¹¹¹. They were first described by Hollicker (1873), who stated that they were the primary agents promoting the activation of bone resorption.

Osteoclasts exhibit various maturation stages with a unique set of morphologic characteristics that determine their resorptive activity^{73,112,113} (Figure 5). They are often found in clusters against the bone surface occupying hollowed-out depressions called Howship's lacunae – shallow channels with an irregular shape that show the cellular activity and mobility during active resorption. Intimately overlapping the bone surface, the cell membrane of the osteoclast presents numerous villus-like enfolded structures, forming the ruffled border (RB). At the border periphery, the plasma membrane is apposed closely to the bone surface.

The cytoskeleton of eukaryotic cells, containing nucleus and organelles enclosed by a plasma membrane¹¹⁴, consists of actin, a family of globular proteins that form filamentous proteins, such as F-actin, actin subunits arranged in a long spiral chain that are responsible for the cell contractile apparatus that supports cytoplasmic constituents and cellular processes (*e.g.*, migration, adhesion, and fusion)¹¹⁵. The adjacent cytoplasm is enriched with proteins associated with integrin-mediated cell adhesion, specifically the $\alpha_v\beta_3$ integrin (*i.e.*, vitronectin receptor) which interacts with bone matrix proteins. Integrins, cellular adhesion proteins, form highly dynamic, cylinder-shaped actin-derived structures called podosomes, mainly identified in pre-mature osteoclasts (pre-OCs)⁷³.

Mature polarized OCs have podosomes further developed into actin rings following complex downstream pathways, which mediate attachment of $\alpha_v\beta_3$ (actin waves) and cytoskeletal rearrangement that results in spreading (podosome clusters), motility (podosome rings), and sealing zone (SZ) assembly (actin belt). The latter is controlled by the external environment; which means that SZ is only formed when OCs are in contact the bone mineral fraction (apatite crystal)¹¹⁶.

Figure 5. A graphic illustration and microphotograph of the microstructural and molecular composition of a multinucleated OC. (a) OCs have multiple nuclei, abundant mitochondria, and a large number of vacuoles and lysosomes. This image was adapted from Takahashi et al. (2014)¹¹² and it is licensed under the Creative Commons Attribution 3.0 Unported License (CC BY 3.0). (b) A transmission electron microphotograph of an OC attached to the mineralized surface of rat bone. OC, osteoclast. This image was adapted from Zhou et al. (2010)¹¹³, ©Elsevier, Inc (license number: 4518171335309).

The SZ alongside with the RB creates the resorption apparatus (RA)¹¹⁷. OC activity and related signaling pathways are described in the following section (see the subsection “remodeling phase”).

Wound-healing phenomena: cell-to-cell communication and healing pathways

Wound healing, specifically peri-implant bone healing, follows a thoroughly organized and sequential tissue repair process following bone trauma due to surgical preparation of the implant site^{118–120} (Figure 6). The osseointegration process implies temporal tissue integration, establishing a contiguous contact at the interface region between the alveolar bone and the implant surface. Understanding of the underlying biomolecular events in wound healing provides a framework to comprehend the biological response arising from host-biomaterial interactions.

Cells respond to varied stimulus in their microenvironment by receiving and processing signals from the ECM. Mechanisms of repair are primarily dependent on cellular cross-talk, from the immune (DCs, M ϕ , T cells) and skeletal systems (MSCs, OBs, and OCs), upon the secretion of a complex array of signaling molecules (ligands; *e.g.*, matrix proteins, growth factors, cytokines and chemokines), which bind to transmembrane receptors – a process termed ligand-binding activity¹²¹.

Essentially, receptor-binding sites activate complex cytoplasmic signaling pathways (*i.e.*, signal amplification), whereupon a complex intracellular cascade of signal transducers phosphorylate certain enzymes while stabilizing others through a highly dynamic and complex process (*i.e.*, transduction). Stable molecules translocate into the nucleus and activate transcription regulators (sequence-specific DNA-binding factors) that control the expression profile of target genes, finally orchestrating the cellular response to an extracellular stimulus¹²² (please see the section “gene expression analysis”).

The spatial and temporal mechanisms of osseous wound healing around implants are similar to the one described during soft tissue and bone fracture healing, wherein bone and the immune system interact intimately¹¹⁸. They consist of four precisely and highly coordinated phases overlapping one another throughout the course of

time, forming a continuous healing process: hemostasis (the exudative phase), the inflammatory phase, the proliferative phase, and the remodeling phase.

Figure 6. Graphic illustrations of the peri-implant bone healing. (a) Hemostasis: platelets perform vascular closure. Growth factors and cytokines are released in the microenvironment; (b) the inflammatory phase: tissue debris and bacteria are phagocytosed by M ϕ . Wound-healing M ϕ 2 limit tissue destruction. In the persistence of bacteria, classically activated M ϕ 1 result in a late inflammatory phase; the proliferative phase: (c) fibroblast migrate through the provisional fibrin matrix to synthesize new collagen and elastin. (d) Perivascular cells are further induced to migrate and promote angiogenesis. (e) Regions of injured pristine bone undergo resorption by osteoclasts to support new bone deposition. (f) Osteoprogenitor cells migrate to the peri-implant interface and bind to the layer of adsorbed proteins, eliciting contact osteogenesis; and (g-h) the remodeling phase: newly deposited woven bone is progressively replaced by lamellar bone, establishing a three-dimensional load-oriented trabecular network around the implant. M ϕ , macrophages. These images were adapted from Terheyden et al. (2012)¹¹², ©John Wiley and Sons, A/S (license number: 4519390064338). Screenshots of the movie “Cell to Cell Communication Osseointegration”: <https://www.youtube.com/watch?v=j4K4sZ4eg-8&t=23s>.

Hemostasis

Upon osteotomy of the implant site, hemostasis begins and continues for minutes to hours. The implant drill damages the bone matrix, cells, blood vessels, and nerves. During drilling, bone debris is formed and may enable the release, dissolution and activation of growth and differentiation factors stored in the bone matrix¹²³. Blood platelets (thrombocytes) further facilitate the liberation of factors while vasoactive agents cause vasoconstriction¹²⁴. However, the distinctive function of platelets during the initial period of osseointegration is not yet well-defined.

Immediately following vascular endothelial damage, bleeding at the injured site allows the biomaterial to interact with ions and water molecules. Released blood matrix proteins (albumin, globulins, fibronectin [Fn], and vitronectin [Vn]) are adsorbed by implant surfaces presenting high free energy^{125,126}, preserving their conformation and function¹²⁷.

Binding of blood matrix proteins to platelets activate their major integrin molecule $\alpha_v\beta_3$, enhancing ligand-binding activity and triggering other cascade events (*e.g.*, platelet aggregation). This mechanism is further strengthened by $\alpha_v\beta_3$ bound to fibrin by generating additional intracellular signaling. Finally, platelets perform vascular closure by aggregation and formation of a thrombus¹²⁸.

Subsequent to protein adsorption, activated thrombin catalyzes the polymerization of insoluble fibrinogen by platelets (extrinsic system) and the intrinsic clotting cascade (Hageman Factor). As a result, the defect is provided with a provisional ECM rich in insoluble fibrin in the immediate vicinity of the implant surface, allowing blood clot stabilization (Figure 6a). Clot retention is a result of binding of platelets to collagen in the fibrin network attached to the surface of the implant through collagen-specific platelet membrane glycoproteins/receptors. This event is complemented by the von Willebrand factor and other glycoproteins, which cause further activation and degranulation of platelets, modulating cellular signaling¹²⁹.

Platelets are an abundant source of growth and differentiation factors (*e.g.*, platelet-derived growth factor [PDGF], insulin-like growth factor [IGF], vascular endothelial growth factor [VEGF], transforming growth factor beta [TGF- β] and fibroblast growth

factor [FGF]). Platelet-released signaling molecules, including cytokine production (*e.g.*, interleukin-1 beta [IL-1 β]), have chemotactic properties that control recruitment, proliferation, and differentiation of diverse hematopoietic and mesenchymal progenitor stem cells^{130,131} responsible for angiogenesis, immune-inflammatory modulation, and bone formation and resorption.

In tandem interplay, the fibrin matrix provides a suitable scaffold for cellular migration, adhesion and proliferation of leukocytes, endothelial cells (inner cell lining of blood vessels) and MSCs (*e.g.*, perivascular cells) to the Ti surface, forming the granulation tissue^{132,133}. Specifically, cell adhesion is strongly influenced by Fn and Vn, which contain cell binding sites that interact with integrins. Cytokines released from degranulating platelets mark the initiation of the inflammatory phase.

Inflammatory phase

As aforementioned, every phase of healing overlaps each other. The inflammatory phase initiates after few minutes following surgery and persists from hours to days, which regulation is suggested to occur in a surface-dependent manner^{134,135}. Inflammatory stimuli result in the release of bradykinin that acts on endothelial cells increasing the blood vessel permeability for fluids, serum proteins, and leukocytes¹³⁶. Histamine, also derived from platelets, induces vasodilatation in replacement of the initial vasoconstriction during hemostasis, increasing the blood flow and resulting in swelling and heating of the local mucosa superimposing the lesion.

The initial inflammatory phase is characterized by activation of the host innate immunological mechanisms by both damage-associated molecular patterns (DAMPs; *e.g.*, heparin and Fn) and pathogen signaling (PAMPs) upon tissue injury and/or cellular stress¹³⁷. The latter consists of non-specific molecules released from bacteria that bind to Toll-like receptors (TLRs) present in the membrane of antigen presenting cells (APCs; *e.g.*, DCs and M ϕ) and further coordinate inflammatory response via different signaling pathways^{138,139}.

Innate immune response to biomaterials involves the cellular defense by PMNs (neutrophils, basophils, and eosinophils), mast cells, natural killers (NK), APCs derived from myeloid progenitors

(DCs and M ϕ), as well as by the complement system (C)^{140,141}. The latter consists of a group of pore-forming cytolytic glycoproteins (perforins), which perforate the plasma membrane of bacteria. Upon the increased blood vessel permeability, leucocytes migrate via the ligand-binding activity of specific molecules throughout the endothelial barrier, facilitated by the action of digestive enzymes (elastase and collagenase). Further migration of PMNs is elicited by chemotaxis exerted by numerous growth factors (*e.g.*, platelet activating factor [PAF] and PDGF), cytokines (tumor necrosis factor alpha [TNF- α] and IL-8), complement 5a (C5a), and other products from fibrin activation and fibrinolysis.

Once the complement is activated, its component 3b (C3b) binds to bacteria glycoproteins directing phagocytosis (*i.e.*, digestion of pathogens, cells debris, and other particles), mainly by neutrophils. Digesting proteolytic enzymes are released from neutrophil granulocytes to eliminate bacteria. These enzymes are toxic for the surrounding cellular components in the wound and may cause tissue breakdown. Increasing granulocyte activity convert plasminogen – an intravascular enzyme with high fibrin affinity – into the active plasmin, generating a proteolytic cascade that contributes to fibrinolysis (*i.e.*, degradation of fibrin)¹⁴². Fibrin is, therefore, vital in hemostasis acting as both the primary product of the coagulation and the final substrate for fibrinolysis¹⁴³.

Macrophages are mononuclear phagocytes considered to be the immune sentinel cells in host reactions to biomaterials by presenting antigens and secreting cytokines and, therefore, directing the processes of homeostasis, wound healing and tissue regeneration¹⁴⁴ (Figure 6b). The macrophage polarization state – the ability of mature macrophages to assume different, mixed phenotypes depending on extracellular cues – determine the immune response owing to the establishment of functionally specialized domains (*e.g.*, signal transduction pathways, and reorganization of the cytoskeleton, organelle, and membrane)¹⁴⁵.

The main phenotypic states of macrophages during wound healing comprise the pro-inflammatory phenotype (classically activated; M ϕ 1) and the wound-healing phenotype (alternatively activated/homeostatic; M ϕ 2). M ϕ 1 are activated by lipopolysaccharides (LPS), an endotoxin found on the outer membrane of Gram-

negative bacteria; and TNF or interferon gamma (IFN- γ), cytokines produced by NK and type 1 Th cells (Th1)^{146,147}. These macrophages are responsible for the phagocytic function and pro-inflammatory component of the immune response, secreting high levels of IL-12 and among other interleukins, for example, IL-1 β , IL-6 and, TNF- α .

In the pursuit of homeostasis, regulatory macrophages (M ϕ regs) further activate Th2 and Treg to produce anti-inflammatory cytokines, which stimulate macrophages to assume a wound-healing phenotype. M ϕ 2 are activated by IL-4 and IL-13 released from Th2, implementing anti-inflammatory functions via production of IL-10¹⁴⁵. They promote wound healing and support tissue repair through the release of TGF- β , PDGF, FGF, and connective tissue growth factor (CTGF), regulating further fibroblast migration through the provisional fibrin matrix into the wound to synthesize new collagen and elastin (Figure 6c).

Under unresolved inflammatory response within the first few hours, increased pyogenic bacterial virulence results in the formation of a purulent exudate containing dead cells and peptide fragments (*i.e.*, pus), initiating the late inflammatory response. Hence, the innate immune response is short-lived and activates the adaptive immunity, highly selective towards specific pathogens and foreign substances¹⁴⁸.

Adaptive immune response comprises two types of mechanisms: the humoral response and the cell-mediated response. The humoral response is given via the production of antibodies by B lymphocytes, which are capable of developing immunocompetence. In turn, T lymphocytes (Th and cytotoxic) are responsible for the cell-mediated response. In this process, Th cells recognize antigens presented by APCs to further recruit B and T cells, whereas the latter kill virus and bacteria.

Secretion of inflammatory cytokines by lymphocytes and macrophages chemoattract fibroblasts, which are further activated by nitric oxide (NO), cyclooxygenase activity and prostaglandin production. Under favorable healing conditions, fibroblasts secrete tissue inhibitors of metalloproteinases (TIMPS)¹⁴⁹; they preserve growth factors stored in the ECM, which concentration is further magnified by the production of other growth factors from M ϕ .

Despite lack of evidence, osteocytes and resident tissue macrophages of bone (osteal macrophages) are suggested to release inhibitory factors that promote tissue regeneration^{150,151}.

As a result of protein adsorption phenomenon, Fn promotes fibroblast migration and adhesion, indicating the beginning of the proliferative phase.

Proliferative phase

This stage of bone healing is mainly characterized by angiogenesis and synthesis of ECM, lasting from days to few weeks post-operatively¹⁵². Fibroblasts migrate into the fibrin clot directed by the concentration gradient of growth factors and the chemoattractant stimulus produced by macrophages, where they synthesize insoluble proteins constituents of the ECM (*e.g.*, COL and other proteoglycans).

Under a hypoxic microenvironment, Mφ further release VEGF, which in addition to FGF, PDGF, and the regulatory effects of TLRs, induce perivascular cells/pericytes (bone-marrow-derived stem cells; BMSCs) to derive endothelial progenitors. The latter migrate to the region of low O₂ concentration where they promote angiogenesis – the growth of a new vascular loop from the existing vascular system –, allowing restoration of a normal blood circulation¹⁵³ (Figure 6d). This process is the prerequisite for new bone formation, which only occurs in a mechanically stable microenvironment in close proximity to blood vessels.

Osteoprogenitor cells at the peri-implant interface bind to the layer of adsorbed proteins on the implant surface through integrin-binding activity, facilitated by additional production of insoluble cellular Fn¹⁵⁴ (Figure 6f). Following cell attachment, bone morphogenetic proteins (BMPs) and other growth factors released from the bone matrix during primary resorption of damaged bone (see the subsection “remodeling phase”) bind to membrane receptors of osteoblast precursor cells, eliciting osteogenic lineage commitment, proliferation, and differentiation^{155,156}.

The master transcriptional regulator during osteogenesis is runt-related transcription factor 2 gene (Runx2), also known as core-binding factor alpha-1 (CBFA-1)^{157,158}, which downstream transcription is regulated by Smad proteins, transducers for extracellu-

lar signals from TGF- β ligands (BMPs and TGF- β), having a wide variety of targets^{100,159}.

Another upstream regulator of Runx2 includes the Wnt-related integration site (WNT) signaling pathways – a group of signal transduction pathways implicated in morphological development and bone modeling^{160,161}. The crosstalk between WNT and BMPs is the major regulatory pathway involved in the transcription of Runx2¹⁶², shown to occur in a surface-dependent manner^{163–166}.

At the early maturational stage of osteoblasts, Runx2 regulates the expression of major bone matrix proteins, such as COL-I, osteopontin (OPN), osteocalcin (OCN), alkaline phosphatase (ALP), bone sialoprotein (BSP). Many molecules, including endocrine factors, serve as coactivators or inhibitors of osteogenesis, including IL-11, parathyroid hormone (PTH), vitamin D, estrogen, calcitonin (CALC), among others, further discussed in more detail¹⁶⁷. Considering that bone always develops by replacement of a pre-existing connective tissue, two different mechanisms of osteogenesis may occur in pre-existing cartilage or directly within the mesenchyme, *i.e.*, endochondral and intramembranous bone healing, respectively⁷³.

WB formation initiates with the secretion of a collagen matrix by OBs, as early as 1 week after implant placement¹⁵², which is rapidly subjected to extrafibrillar mineralization by hydroxyapatite in an unorganized fashion, oriented parallel to the implant surface¹⁶⁸. WB formation originates from both the host bone by forming bridges throughout the bone defect, as well as in direct contact to the implant surface – so-called distance and contact osteogenesis, respectively.

Remodeling of bone in direct contact to the implant and subsequent new bone formation depict the beginning of the remodeling phase¹⁵², suggested to last from weeks to months in humans.

Remodeling phase

Physiological bone metabolism and the skeletal system are linked to phosphate and calcium, which normal concentrations are contingent upon mechanical (compression and shear stresses, and tensile strength) and/or biochemical signals (metabolic and nutritional

stress). Bone remodeling is, therefore, a complex versatile process related to the three broad functional roles of the skeletal system – mineral hemostasis, hematopoiesis, and mechanical through adaptation to overloading stress and healing of microfractures and bone necrosis¹⁶⁹. Thus, enhanced peri-implant bone healing and long-term clinical success of osseointegration is a result of a rather functional balance between cellular cross-talk (MSCs, OBs, and OCs) than complete inhibition of OC activity. Specifically, the rate at which secondary stability occurs is dependent on the mechanism of coupling bone resorption and formation.

Upon implant insertion, the static strain initially responsible to ensure primary stability is released owing to the relaxation effect of the bone¹⁷⁰. Simultaneously to osteoblastic differentiation and maturation, regions of injured pristine bone in intimate contact to the implant surface (host BIC [h-BIC]) undergo resorption to create adequate space over its critical threshold strain and to support new bone deposition^{152,171} (Figure 6e). This healing mode is termed interfacial remodeling and, as a result thereof, a transient implant stability drop occurs after a few weeks of implantation^{172–175} (Figure 7). This event is especially critical when surgical instrumentation and placement of an implant generate frictional forces and lateral compression beyond the yield strain of the hard tissue¹⁷⁶.

The origination phase begins with the conversion of a quiescent bone surface into a remodeling surface with the recruitment of preosteoclasts. As previously discussed, osteoclasts originate from multipotent hematopoietic stem cells, which lineage commitment to bone marrow precursors is dependent on the microphthalmia-associated transcription factor (MITF) and PU.1, following stress, injury, bacterial and viral infection^{177,178}. After that, macrophage colony-stimulating factor (M-CSF), secreted by MSCs and OBs, is responsible for primary differentiation of Mo/M ϕ into preosteoclasts¹⁷⁹.

During this process, temporary functional compartments containing basic multicellular units (BMUs) are formed at distinct focal anatomical sites without damaging the surrounding environment (Figure 8). The BMU is constituted of the vasculature that brings nutrition and aggregates of osteoblasts, osteoclast lineage cells, and their end-stage phenotypes¹⁸⁰. These bone remodeling

units emerge in close proximity to a blood vessel, both in cortical and cancellous bone in the form of tunnel-shaped structures or in contact with the endosteal surface of trabeculae, respectively. The sequence of events, controlled by the formation-resorption coupling mechanism, consisting of five phases: origination, resorption, reversal, formation, and termination.

Figure 7. A graphic illustration of the development of primary (mechanical) implant stability into secondary (biological) stability during healing (in weeks) as assessed by RFA. Upon implant placement, regions of injured pristine bone in intimate contact to the surface, initially responsible to ensure primary stability, undergo resorption to support new bone deposition (secondary stability). This mechanism results in a transient total implant stability dip, as suggested to occur in humans after a few weeks of implantation¹⁷³. The predictive values of RFA are reported in accordance with Baltayan et al. (2016)¹⁷⁴. RFA, Resonance Frequency Analysis. This image was adapted from Suzuki et al. (2013)¹⁷⁵, ©Lippincott Williams & Wilkins (license number: 4565420330232).

Finally, DNA transcription results in activation of a widespread array of target genes responsible for cytokine production, modulating osteoclastogenesis, osteoclast survival, and activity. Conversely, cellular differentiation, fusion, and activation are directly inhibited by osteoprotegerin (OPG), an osteoblast-secreted soluble glycoprotein that binds to RANKL, preserving bone^{181,182}.

Figure 8. A graphic illustration of a BMU and sequential events of the coupling mechanism during remodeling. Longitudinal section through a cutting/filling cone in the cortical bone (moving to the right) illustrating the perivascular cellular activity associated with the coupled resorption (R) and formation (F) responses during remodeling. Four cross-sections of the evolving secondary osteons are shown throughout the progression of resorption to form a cavity within the cortical bone to refilling it with lamellar bone is shown from left to right. Color code: black, T lymphocytes; red, osteoclast cell line; blue, osteoblastic line; green, the reversal cement line formed at the peripheral limit of the osteon. BMU, bone multicellular unit. This image was reproduced from Roberts et al. (2003)¹⁷⁷, ©Elsevier, Inc (license number: 4518220633316).

Thereupon, osteoclastogenesis depends on the activation of the key regulatory molecule, receptor activator of nuclear factor kappa B (RANK)¹⁸³, by its insoluble membrane-bound ligand (RANKL) – members of the TNF superfamily secreted by stromal cells (MSCs, OBs, DCs, and osteocytes) and Th17^{108,184–187} (Figure 9). RANK transduces signals by recruiting adaptor molecules, for instance, TNF receptor-associated factor (TRAF-6)¹⁸⁸. This molecule, in conjunction with other intracellular transducers, allows activation of the mitogen-activated protein kinases (MAPKs), the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and the activator protein 1 (AP-1) mechanisms¹⁸⁹. Activation of AP-1 occurs through the induction of c-Fos which,

among other proteins, is dependent on the phosphorylation of c-Jun by MAPKs and on the activation of NF- κ B (Figure 10).

Figure 9. A detailed illustration of hematopoietic precursor cell differentiation into mature OCs. Maturation occurs from bone marrow precursors committed to Mo/Mφ cell lineage by stimulation with CFU-GM. M-CSF and RANKL are essential for primary differentiation of Mo/Mφ into pre-OCs and for complete osteoclastogenesis. OPG binds and neutralizes RANKL, and negatively regulates both osteoclastogenesis and activation of mature OCs. CFU-GM, granulocyte-macrophage colony-stimulating factor. M-CSF, macrophage colony-stimulating factor. Mo/Mφ, monocyte/macrophage. OCs, osteoclasts. OPG, osteoprotegerin. RANKL, receptor activator of nuclear factor kappa B ligand. This image was adapted from Boyle et al. (2003)¹⁷⁷, ©Nature Publishing Group (license number: 4565310836449).

Once the adaptive immune-inflammatory response is triggered, cellular release of the central insoluble RANK causes an alteration in the RANKL/OPG ratio, resulting in activation of RANK¹⁹⁰. During the resorption phase, OC activity creates cutting cones in the anterior region of the remodeling unit through activation of functional membrane structures. BLCs, located in close contact with osteoclasts, are suggested to play an essential role in the progression rate of BMUs towards its target (25-30 μm/day longitudinally and 6-7 μm/day radially in humans) as they express both M-CSF and RANKL¹⁹¹.

Figure 10. A diagram of the canonical and non-canonical NF-κB signaling induced by RANKL and TNF, respectively. RANKL and TNF induce canonical signaling by recruiting TRAF6 and TRAF2/5, respectively, to their receptors to activate a complex consisting of IKK-α, IKK-β and IKK-γ (NEMO), which induces phosphorylation and degradation of IκB-α and the release of p65/p50 heterodimers, which translocate to the nucleus. This induces expression of c-Fos and NFATc1 as well as the inhibitory κB protein, NF-κB p100. In unstimulated cells, p100 binds to RelB to prevent its translocation to the nucleus. RANKL induces the ubiquitination and lysosomal degradation of TRAF3 through TRAF2/cIAP1/2, releasing NIK to activate IKK-α, which leads to proteasomal processing of p100 to p52; RelB:p52 heterodimers then go to the nucleus to induce target gene expression. TNF does not degrade TRAF3, and thus NIK is degraded, leading to the accumulation of p100 in the cytoplasm of osteoclast precursors to limit their differentiation. cIAP1/2, cellular inhibitor of apoptosis 1/2. IKK, IκB kinase. NEMO, NF-κB essential modulator. NIK, NF-κB-inducing kinase. NFATc1, nuclear factor of activated T-cells, cytoplasmic 1. NF-κB, nuclear factor kappa B. RANKL, receptor activator of nuclear factor kappa B ligand. RelB, proto-oncogene, NF-κB subunit. TNF, tumor necrosis factor. TRAF6, TNF receptor (TNFR)-associated factor 6. This image was adapted from Brendan et al. (2015)¹⁸⁹ and it is licensed under the Creative Commons Public Domain Mark 1.0.

During osteoclast activity, cell-matrix interactions occur resulting in the release of hydrogen ions in the sealed compartment through the activity of vacuolar-type H⁺-ATPase and carbonic anhydrase II (CA2, a metalloenzyme that catalyzes the hydration of carbon dioxide [CO₂] and the dehydration of bicarbonate)¹⁹², while chloride channels provide a Cl⁻ flux. The SZ ensures isolation of an acidic microenvironment (pH ~4.5) for bone demineralization and organic matrix exposure through a defined concentration of lysosomal proteolytic enzymes. In the resorption lacunae, they release enzymes and collagenase into the sealed compartment in the osteoclast-bone interspace, essentially creating an extracellular lysosome.

Acid phosphatase (AP) is an abundant plasma membrane-bound glycoprotein that catalyzes the hydrolysis of phosphate monoesters at basic pH values. OCs, as well as activated Mφ, synthesize the type 5b acid phosphatase (Tartrate-Resistant Acid Phosphatase; TRAP) – a metalloproteinase resistant to the inhibitory effect of L(+) tartrate on the catalytic activity of other acid phosphatases^{193,194}. Therefore, increased TRAP synthesis indicates osteoclast formation through Mφ fusion and further activation, which distinguishes OCs from multinucleated giant cells (MNGCs) in addition to an opposing signaling pathway in cellular differentiation.

Simultaneously with TRAP activity, additional proteolytic enzymes, such as matrix metalloproteinase-9 (MMP-9) and cathepsin-K (CTSK), are secreted into the resorption lacunae and result in respective mineral and organic bone matrix cleavage and degradation^{195,196}. MMP-9 removes the superficial collagenous layer to enable initial bone degradation and eliminates the remaining collagen in the resorption pits following CTSK digestion. Products derived from matrix degradation (*i.e.*, organic fragments and mineral ions) are then endocytosed at the central portion of the RB and released through transcytosis by the functional secretory domain (FSD) located at the OC membrane¹⁹⁷.

Finally, PTH and vitamin D basal levels are responsible for controlling calcium phosphate homeostasis and bone mineralization. This vitamin positively modulates osteogenic response, optimizing bone remodeling through reduction of systemic bone

resorption^{198,199}. PTH has the opposite effect, controlling osteoclastic activity by increasing RANKL and lowering OPG release by osteocytes²⁰⁰. Secretion of this hormone is downregulated by vitamin D and increased by the low concentration of blood Ca^{2+} levels. Nevertheless, vitamin D also stimulates bone resorption in order to balance bone homeostasis by altering the RANKL-OPG ratio, although the understanding of its effects during the process of osseointegration and its maintenance remains limited²⁰¹. In turn, calcitonin and estrogen, antagonistically to PTH, inhibit bone resorption by lowering the levels of Ca^{2+} and PO_4^{3-} .

On the basis of anti-resorptive mediators, physiological levels of CALC, a hormone produced by thyroidal parafollicular cells, present a wide range of important roles that act antagonistically to PTH²⁰². Besides promoting collagen synthesis and osteogenic differentiation of progenitor cells, CALC regulates calcium homeostasis during bone remodeling through regulation of its receptor (CALCR) present in osteoclasts²⁰³. CALCR is expressed in TRAP-positive cells, but not in all pre-OCs, and is directly proportional to the blood level of calcitriol (1,25-dihydroxyvitamin D3 [$1\alpha,25\text{-(OH)}_2\text{D}_3$]), the hormonally active metabolite of vitamin D.

In addition, recent studies have identified the anti-osteoclastogenic activity of enzymes involved in polyamines synthesis (arginases), responsible for various cellular functions comprising growth, differentiation, and survival. Specifically, ARG1 is a well-known biomarker of M ϕ 2 and its expression is upregulated during phagocytosis, osteoclast differentiation, and bone resorption; while its knockdown enhances formation of mature OCs²⁰⁴. This enzyme mediates osteoclastogenesis negative modulation by limiting l-arginine for production of nitric oxide (NO).

Following bone resorption, the reversal phase consists of a transitional period where bone resorption is negatively modulated by an increase in OPG and then coupled with the mechanism of bone formation²⁰⁵. BLCs migrate to Howship's lacunae and digest the non-mineralized matrix by releasing MMPs. Subsequently, deposition of a thin layer of collagenous matrix forms a rich in OPN cement line, which facilitates OBs attachment and deposition of new osteoid tissue.

Both preosteoblasts and osteoblasts produce high levels of ALP on the outer surface of their plasma membrane. Even though the role of ALP in bone-forming cells is rather complex and not yet completely well-defined, it is known to be involved in the cleavage of organically bound phosphate (*i.e.*, dephosphorylation), thereby initiating bone mineral deposition throughout precipitation of apatite. At the mineralization front, BSP is found within the spaces between the calcified collagen fibrils²⁰⁶.

Once newly WB is deposited, osteogenic tissue is progressively replaced by load-bearing LB. Thereafter, interfibrillar mineralization occurs following bone remodeling by the formation of uniaxially oriented plate-shaped hydroxyapatite crystals at the nanometer level²⁰⁷, resulting in trabeculae oriented similar to a buttress and ensuring a long-term secondary stability (Figure 6g-h). During the termination phase, osteoblasts become quiescent and flattened BLCs, responsible for continuous intercellular communication with osteocytes.

Prognostic factors and long-term maintenance of the peri-implant bone support

As aforementioned, implant failure occurs in connection with multifactorial conditions at the patient and implant levels²⁶. Early failure (*i.e.*, unsuccessful osseointegration, indicating impaired bone healing) is associated with several local factors (*e.g.*, low-density bone [type III and IV], compromised bone volume, implant micromotion, immediate implant placement in fresh extraction sockets, bone overcompression, titanium allergy and tobacco use)²⁰⁸, systemic diseases and associated disorders (rheumatoid arthritis combined with connective tissue disease²⁰⁹, uncontrolled diabetes mellitus^{210,211}, radiotherapy²¹², inflammatory bowel disease [Crohn's disease]²¹³, and medication-related risks (antidepressants and proton pump inhibitors)²¹⁴⁻²¹⁶ that interfere with bone wound healing and remodeling. In addition, implant surface properties (*e.g.*, a machined surface), implant macrogeometry (*e.g.*, a cylindrical non-threaded implants), and the operator experience also appear to affect the incidence of early implant failure^{217,218} (see the section "strategies to enhance bone deposition and shorten healing times").

On the other hand, late implant failure (*i.e.*, loss of previously established osseointegration) may occur as a result of peri-implantitis (an inflammatory disease with progressive loss of supporting alveolar bone beyond the physiologic bone remodeling)²¹⁹, heavy tobacco usage²²⁰, bruxism^{26,221}, and the aforementioned systemic disorders²²². Shorter-length implants may also negatively affect implant failure rates²²³.

With regard to the host site, correct implant placement requires an adequate bone volume, from a functional and aesthetic outlook. The alveolar process is a tooth-dependent anatomic structure resting on basal bone²²⁴. Upon tooth extraction, alveolar ridge dimensional changes occur in post-extraction sites due to extensive bone resorption, which results in loss of volume and change in ridge contour^{225,226} in spite of applying preservation techniques²²⁷. Reportedly, 11-22% of the vertical dimensional changes and 29-63% of the horizontal bone loss, the latter being more pronounced in the buccal aspect of the extraction socket, occurs between 6-7 months post-operatively²²⁸. Furthermore, complete cortication in 80% of extraction sockets in humans only occurs after a considerably long period of time 9-12 months²²⁹.

The extent of dimensional changes is affected by variable circumstances, for example, the number of extracted teeth and residual bone walls, bone mineral density (*i.e.*, stiffness), the severity of infection, and so forth²³⁰. Severely deficient alveolar ridges are found in an apical level and lingual/palatal position with an extensive reduction in the quantity and architecture of bone, and changes to the soft tissue contours (*e.g.*, lack of buccal convexity). Such alveolar process deficiency significantly limits implant therapy both in the upper and lower jaw. This is particularly vital in the management of severely resorbed maxillary and mandibular ridges due to not only insufficient volume but also low-density and poor-quality bone (structural morphology and cellular composition).

Success criteria for oral implants have been previously proposed by several authors²³¹⁻²³⁶. The criterion suggested by Albrektsson et al. (1986)²³⁷ is the one most commonly applied, comprising five aspects: immobility; absence of radiolucency; no signs or symptoms (*e.g.*, infections, lesions, pain, etc.); CBL less than 1.5 mm after 1 year of functional loading and 0.2 mm annually thereafter; and,

finally, 85% and 80% success rates after a period of 5 and 10 years, respectively. For instance, Misch²³⁶ suggested that implants presenting pain on function, mobility, radiographic CBL of more than one-half the length of the implant body and uncontrolled exudate are suggested to be removed.

According to recent literature, a healthy peri-implant site is absent of erythema, profuse bleeding on probing (BoP) under light forces (< 0.25 N), swelling, suppuration, and CBL beyond bone level changes resulting from initial bone remodeling as a result of surgical trauma (CBL less than 2 mm after the first year in function)²³⁸. Clinical signs of inflammation are considered more relevant than defining a range of probing pocket depth (PPD; ideally ≤ 5.0 mm). However, an increase in PPD over time compared to baseline must be interpreted as pathologic. Hence, evaluation of MBL is a significant measure for the assessment of implant success (Figure 11)²³⁹, of special importance in the presence of clinical signs of inflammation considering that healthy implant sites are possible to exist despite reduced bone support.

Therefore, visual evidence of inflammatory status, BoP and/or suppuration, increasing PPD, and progressive bone loss, represent a clinical condition of peri-implantitis^{219,240}. In the absence of baseline radiographs and PPD, peri-implantitis is otherwise detected based on bone loss ≥ 3 mm and/or PPD ≥ 6 mm in association with BoP.

Figure 11. Radiographic measurement of interproximal and peri-implant MBL. DCB, distance from the contact point to the most coronal point of the bone crest. DIB, distance from the implant shoulder to the first visible bone-to-implant contact. MBL, marginal bone level. This image was adapted from Molina et al. (2017)²³⁹, ©John Wiley and Sons, A/S (license number: 4584131510162).

STRATEGIES TO ENHANCE BONE DEPOSITION AND SHORTEN HEALING TIMES

The management of challenging clinical scenarios aims to achieve sufficient bone for implant placement and restoration of functional loading by means of various reconstructive surgical techniques. Among them, block bone grafting, alveolar distraction osteogenesis, ridge splitting/expansion, and maxillary sinus floor augmentation (MSFA) have been proven to yield proper bone regeneration²⁴¹. Novel strategies include the principles of guided bone regeneration (GBR) – the reconstitution of a lost or injured tissue structure and function based on the potential of osteoprogenitor cells to proliferate and differentiate in the osseous wound²⁴² – and may be applied prior to or in conjunction with implant therapy to reestablish the facial bone wall thickness, especially in the aesthetic zone²⁴³.

GBR includes bone tissue engineering (BTE) strategies that apply extracellular elements to attain osteoconductivity – the process whereby a 3D scaffold or matrix provides osteoblast migration and new bone formation on its surface²⁴⁴. BTE consists of endogenous regenerative technology (ERT; *e.g.*, cellular elements and/or key endogenous molecules) applied in combination with scaffolds to achieve osteoinductive properties – the process by which MSCs recruitment and differentiation to bone-forming cells are induced²⁴⁵ –, offering a greater regenerative capacity to biomaterials. Among different indications, they attempt to minimize the loss of alveolar bone after extraction in case of favorable residual bone volume and

height, to augment deficient alveolar ridge width and height, and to regenerate peri-implant bone defects (*e.g.*, fenestration and dehiscence) and critical size cranio-maxillofacial defects^{241,246}.

Thus, the definitive purpose of contemporary research and innovative industry is to engineer implant surface technologies and hardware design that positively modulate early wound healing and enhance osseointegration whether in resident bone or augmented sites, consequently achieving high mechanical performance in different clinical scenarios^{247–249}. In this context, implant surface modifications, implant macrogeometry, and surgical techniques have been developed during recent years to enhance osseointegration and to minimize treatment timeframes in order to apply functional loading.

Implant surface modifications

The high survival and clinical success rates of contemporary implants are based on, among other factors, progressive advances in oral implant surface technology²⁵⁰ – the most studied parameter in the last four decades. Surface modifications include topographical, chemical, physical and mechanical alterations, constituting a complex hierarchical structure. Altogether, they result in biomimetic topographical features, chemical composition, and physical properties (charge and wettability)^{251,252}, which affect surface bioactivity (*i.e.*, potential to form bioactive bonding with bone by forming an apatite layer at the bone-implant interface)²⁵³.

Topographical properties

Topographical properties are described as alterations on the surface outmost layer in the micrometer length scale that provide osteoconductivity by mimicking the cellular microenvironment^{254–257}. Microstructures resemble underlying features found on the bone following resorption by osteoclasts. Through acidification and proteinase activity, the RA of OCs produces microscale pits (up to 100 x 50 µm, diameter x depth) with roughness at the submicron length scale^{258,259}. The residual collagen fibers offer nanostructured cues that correspond to the sizes of proteins and cell membrane receptor, directly affecting osteoblast phenotypic expression²⁶⁰.

Implant surface morphology and microtopography can be evaluated by means of scanning electron microscopy (SEM), interferometer (INF), and confocal laser scanning microscopy (CLSM). Nanotopographical features are analyzed by means of atomic force microscopy (AFM)²⁶¹. The attained surface roughness is assessed by height, spatial, and hybrid parameters²⁶². Specifically, average height amplitude (R_a), a two-dimensional (2D) value, has been assessed and categorized modified surfaces into the following degree of roughness¹⁹:

- $R_a < 0.5 \text{ }\mu\text{m}$: smooth or machined surfaces
- $R_a = 0.5 - 1.0 \text{ }\mu\text{m}$: minimally rough surfaces
- $R_a = 1.0 - 2.0 \text{ }\mu\text{m}$: moderately rough surfaces
- $R_a > 2.0 \text{ }\mu\text{m}$: rough surfaces

Other commonly reported 3D height parameter of surface roughness includes the shape of the amplitude distribution curve (S_{sk}). Spatial measurements describe the lateral property of the surface and include the texture aspect ratio (S_{tr}), indicating the presence of anisotropic properties (physical features without a prevailing direction), and the number of peaks per area unit (S_{ds}). Finally, hybrid parameters describe the shape of the surface by measuring the mean slope and the developed area ratio (S_{dr})²⁶¹.

Initially, implants were manufactured by means of a turning process, resulting in relatively smooth surfaces with clearly oriented grooves (*i.e.*, isotropic surfaces). In spite of machined implants allowing osseointegration, relatively long waiting times were needed prior to applying occlusal loading, according to the protocol suggested by Brånemark⁴⁰. With the attempt to surpass the biological response to machined surfaces, titanium plasma sprayed (TPS) implants were developed to generate rougher surfaces ($R_a = 4 - 5 \text{ }\mu\text{m}$). Despite increased osseointegration shown in *in vivo* preclinical models²⁶³, TPS implants were more susceptible to bacterial plaque accumulation, thereby increasing the frequency of peri-implantitis²⁶⁴, compared to minimally and moderately rough surfaces²⁶⁵. Therefore, they are no longer commercially available.

Innovative manufacturing procedures that yield moderately roughness promote enhanced BIC and higher resistance to removal torque (RTV; *i.e.*, interfacial shear strength) in comparison to min-

imally rough machined surfaces^{266,267}. Moderately rough surfaces are shown to increase platelet activation and improve fibrin retention²⁶⁸, and positively regulate early cell migration, attachment, proliferation, and polarity^{269,270}, resulting in increased levels of BIC and biomechanical fixation^{271,272}. This phenomenon is termed contact guidance by which the biomaterial provides directional cues to the cells, directing migration response associated with structural anisotropy²⁷³.

Lastly, surface biofunctionalization at the nanometer length scale (1 – 100 nm) in combination with micron-/submicron-scale roughness has recently been introduced by applying thin functional coatings or following chemical treatment and storage in saline solution²⁷⁴. Several studies have shown the potential of nanostructured surface topography to influence the early biological response by modulating macrophage activation towards M ϕ 2^{135,275,276} and osteoblast-related gene expression^{277–279}.

Furthermore, nanotopography has shown positive effects on detachment of oral biofilm around the implant collar surface²⁸⁰, thereby contributing to prevent infectious diseases that trigger the immune-inflammatory host response (peri-implant mucositis and peri-implantitis).

Physical and chemical properties

Surface free energy and wettability

The surface energy of a biomaterial subsists at different levels depending on the charge density (amount of ionic concentration per surface area) and net polarity of the charge (number of opposing electric charges according to the distribution of ions, negative or positive polarity)²⁸¹. The outermost native oxide layer of Ti implants is highly responsive to chemical reactions²⁸². Since the moment of exposure to atmospheric air, a Ti implant is subjected to a time-related reduction in its biocompatibility due to contamination with, for example, hydrocarbons and carbonates, termed “ageing”. The importance of sterilization was initially demonstrated by applying the technique of radiofrequency glow-discharge (RFGD)^{283,284} – a plasma formed by the passage of electric charge through an ionized gas under vacuum, highly effective to remove

hydrocarbon layers. Thus, the concept of cleanliness was the primary basis of generating high surface free energy.

Certain treatments, for example, sandblasting and acid-etching²⁸⁵, form intermolecular hydrogen (H) bonds with the free oxygen atoms. Following manufacture and rinse with an aqueous solution under nitrogen (N) protection, which prevents contamination from the beginning, nucleophilic hydride layers are formed on the surface – a process termed hydroxylation²⁸⁶. Further maintenance of hydrophilicity was shown to be possible following immersion in sterile triply distilled water²⁸⁷, which was later revealed efficient to retain the surface hydrophilicity and hydration. Today, this method includes storage in a sealed glass tube containing sodium chloride (NaCl) solution¹²⁶.

By increasing the static electric charge (polarity) on a surface, its wettability, *i.e.*, the ability of one fluid to spread on or adhere to a solid surface, is also increased owing to the negative charge of hydroxyl groups (hydroxide; OH⁻). By definition, materials with a special affinity for water (H₂O), a polar molecule, are known as hydrophilic, while hydrophobic materials lack such affinity²⁸⁸. Static and dynamic water contact angle (θ) measurements are employed to analyze the level of wettability related to the surface tension (Figure 12a). Contact angles define a surface as hydrophobic ($\theta > 90^\circ$) and hydrophilic ($\theta < 90^\circ$; Figure 12b).

When a chemically modified hydrophilic implant is in direct contact with blood, the entire surface is immediately covered by the fluid (Figure 12c). Adsorption of blood and molecules create an electric field on the TiO₂ layer, causing titanium oxidation. This charge regulation reduces the potential of dissolution, and thereby disturbs molecular interchain reactions that prevent ionic interactions of cellular and plasma proteins (*e.g.*, Fn) to the surface. As a result, mechanisms of biochemical binding follow changes in the conformation, adsorption, and function of blood integrin-binding proteins^{289,290}. This mechanism increases protein concentration by a factor of 1000 compared with hydrophobic surfaces¹²⁷. Hence, hydrophilic surfaces allow fluid and ions exchange between biomaterial and body fluid, controlling protein adhesion and cell activity²⁹¹ (Figure 13a).

Figure 12. Diagrams showing the static contact angle (θ) of a liquid droplet on solid surfaces with different wettability. (a) The graphical derivation of Young's equation. **(b)** Correlation between wetting tension and the wetting of a solid. These images were adapted from Rupp et al. (2014)²⁴⁸, ©Acta Materialia, Inc. Published by Elsevier, Ltd (license number: 4519370658151). **(c)** Implants with high wettability (hydrophilic) present a greater static electric charge than the one of the wetting liquids. Hydrophilicity ensures more rapidly surface coverage with blood. **(d)** Implants with low wettability (hydrophobic) present a low surface tension. These images were provided by Neodent®, Curitiba, PR, Brazil.

Accordingly, hydrophilic surfaces provide the local environment with blood clot stabilization, which regulates molecular and cellular events towards immune-inflammatory modulation^{275,292} and osteogenesis^{126,293,294}, stimulating superior host-to-implant interactions and early deposition of new bone²⁹⁵⁻²⁹⁹. On the contrary, hydrophobic surfaces allow the air to be entrapped in its interface when exposed to a wetting liquid, which may lead to protein denaturation through exposure to hydrophobic amino acid residues³⁰⁰ (Figure 12d and 13b). This interferes with plasma protein adsorption processes and signaling pathways that support early wound healing. Accordingly, low surface free energy and increasing hy-

drophobicity³⁰¹ have been shown to negatively affect osteoblast attachment and spreading, alkaline phosphatase activity, and calcium mineralization³⁰².

Figure 13. Graphic illustrations of the possible cell-to-surface interactions on hydrophilic vs. hydrophobic surface technologies at different length scales. (a) Hydrophilic surfaces achieve rapid coverage of the implant surface with blood and biological fluids, allowing normal protein adsorption in a configuration that exposes adhesion motifs, enhancing interactions with cell receptors (e.g., integrins). **(b)** Hydrocarbon contamination in hydrophobic surfaces causes denaturation of proteins by disturbing their tertiary structure which prevents cell adhesion. These images were reproduced from Gittens et al. (2014)²⁴⁹, ©Acta Materialia, Inc. Published by Elsevier, Ltd (license number: 519331146310).

Bioactivity

Biomimetic approaches comprise engineered thin coatings at the nanometer length scale aiming to deliver functional molecules with biomimetic properties^{32,303–306}. The most common modifications of a surface chemical composition include implementation of mineral ions, such as Ca, Mg, HA, and F, among other recent technologies further discussed in the next section.

These surface technologies develop high surface energy and hydrophilicity with high potential to positively modulate the biological response. Correspondingly, enhanced osseointegration, in terms

of faster and larger amounts of BIC³⁰⁷ and nanomechanical properties³⁰³, is achieved by bioactive surfaces in comparison to machined and micro-rough surfaces lacking nanotechnology, as shown in preclinical experimental studies; however, their long-term clinical efficacy remains to be evaluated. Surface chemical composition and thickness of the oxide layer are measured with x-ray photoelectron spectroscopy (XPS)²⁶¹.

Additive vs. subtractive manufacturing of oral implants

The most commonly used methods to manufacture micro-rough surfaces consist of additive and subtractive processes, both aiming to enlarge the surface contact area^{252,308}. The former manufacturing methods include the addition of ceramic^{309,310} or metal onto the surface, creating bumps (*e.g.*, TPS; Figure 14a)²⁶³. In turn, subtractive processes remove particles from the surface, creating pits and grooves (*e.g.*, machined surface; Figure 14b). The most common procedures are sandblasting, acid-etching by mineral acids, electrochemical anodic oxidation, laser modification, CaP coatings, or combinations thereof²⁵¹. When produced under favorable conditions, several of these methods allow an increase in surface free energy and thereby hydrophilicity. Otherwise, the manufacturing process to obtain a moderately rough topography will result in hydrophobic implants.

Acid-etching (AE) has been applied as a surface treatment with varied solution concentrations, temperature, and duration³¹¹. Specifically, dual AE produces a textured topography characterized by pores of different shapes and defined edges (Figure 14c). This treatment may include solutions containing hydrochloric acid (HCl) and sulfuric acid (H₂SO₄)³¹², or a mix of hydrofluoric acid (HF) and nitric acid (HNO₃)³¹³. In addition, other techniques might be applied in combination with double acid-etching to obtain optimal levels of roughness and surface free energy, such as blasting^{312,314–316} or anodic oxidation^{317–319}.

Blasting techniques are performed with different media containing particles within the micron size range (*e.g.*, 25- and 75- μ m sized Al₂O₃, *e.g.*, corundum [SLA¹²⁶; Figure 14d], resorbable bio-compatible ceramics [resorbable blasting media, RBM³²⁰; Figure 15a] or TiO₂³²¹ [Figure 15b]) under compressed air. The shape and

size of the particles, distance of the implant to the nozzle (~10 mm), duration (10-15 min) and pressure (*e.g.*, 380 kPa) during treatment are the main factors that control the level of roughness and surface energy. Nevertheless, following these processes, a layer of compressive residual stress is created due to the formation of crystal defects³²². Hence, sandblasted surfaces are further acid-etched in order to remove deformed atomic layers and remaining particles, optimizing the level of surface roughness and energy.

Figure 14. Scanning electron micrographs of implant surfaces treated by means of different manufacturing procedures. (a) A TPS processed surface (Straumann®, Basel, Switzerland) depicting the cracks inherent to processing due to rapid cooling. **(b)** A machined surface with clearly oriented grooves. **(c)** A dual acid-etched surface (Biomet-3i, Palm Beach Gardens, USA). **(d)** SLA surface (Straumann®, Basel, Switzerland). SLA, sandblasted, large grit, acid-etched. TPS, titanium plasma sprayed. These images were reproduced from Coelho et al. (2009)³⁰⁸, ©Wiley Periodicals, Inc (license number: 4526651462268).

In particular, the biological effect of a moderately rough sandblasted, large grit (250 - 500 µm corundum grit), acid-etched surface with HCl and H₂SO₄ (SLA) has been extensively reported in both preclinical and clinical studies. SLA contains micro-topographical

features (20- to 40- μm -wide cavities), produced by the blasting process, overlaid with etched microstructures (0.5 - 3 μm in $\varnothing$) comparable to osteoclastic resorption sites on bone^{260,285}. Despite its hydrophobicity, SLA results in better BIC and higher RTV at early stages of bone healing (up to 3 months), compared to minimally rough machined and TPS surfaces^{271,323,324}. The potential of achieving a greater and better quality of osseointegration over time with this surface treatment allows immediate or early functional loading of the implants^{325–328}, with excellent results reported at sites presenting bone type IV^{267,329}. In addition, high levels of clinical success with a minimal CBL on the long-term were recently reported for implants with this type of surface technology³³⁰.

Figure 15. Scanning electron micrographs of implant surfaces treated by means of different manufacturing procedures. (a) A RBM-treated implant surface (Biolog-Biohorizons, Birmingham, USA). **(b)** A titanium oxide blasted/acid-etched implant surface (TiOblast®, Astra, Mölndal, Sweden). **(c)** A PSHA-coated surface (Bicon LLC, Boston, USA). **(d)** An anodized surface (TiU-nite®, Nobel Biocare, Sweden). PSHA, plasma-sprayed hydroxyapatite. RBM, resorbable blasting media. These images were reproduced from Coelho et al. (2009)³⁰⁸, ©Wiley Periodicals, Inc (license number: 4526651462268).

Nanometer topography may also be achieved by applying a nanoscale coating to the implant surface following a variety of fabrication methods, such as sand-basting and acid-etching³⁰³, laser deposition³³¹, UV irradiation³³², plasma-immersion³³³, among others. Some of the contemporary methods to create chemically stable and bioactive surfaces may include functionalization with biochemical nanocoating by applying TiO₂ films, ceramic or protein (*e.g.*, mesoporous TiO₂, HA (Figure 15c), laminin-1, and bioactive glass)^{32,334–336}, local drug (*e.g.*, bisphosphonates, clarithromycin, and strontium [Sr] ranelate) and chemical compound (*e.g.*, BMPs, Mg, CaP, and fluoride [F]) release systems^{30,337}, and development of biodegradable implants³³⁸, regarded as next-generation oral implants.

Anodic oxidation improves tissue response to implants by modifying the crystal structure and thickness of the TiO₂ layer³³⁹ (Figure 15d). This technique is based on the electrochemical process, where the implant functions as the anode (the electrode through which the electrical current enters). Anodized surfaces may receive, for instance, incorporation of CaP³⁰⁴ or Mg³⁴⁰ onto the surface, commonly following acid-etching treatment. This method significantly increases the surface porosity, hydrophilicity, and resistance to chemical dissolution³²². Specifically, Ca-incorporated oxidized implants increase the amount and rate of BIC in *in vivo* models, compared to both machined and moderately rough hydrophobic implants surfaces^{304,341}.

Chemically modified hydrophilic implant surfaces

As previously stated, clean and hydrated surfaces allow the formation of a highly energetic state characterized by stable chemical compounds, which results in high hydrophilicity. In addition to preserving activated surface states, other engineering approaches that result in increased implant surface hydrophilicity may include alkaline treatment³⁴², plasma cleaning^{343,344}, polyelectrolyte modification³⁴⁵, ultraviolet (UV)-C treatments³⁴⁶ and UV-A activation of photocatalytic films³⁴⁷ (Figure 16).

Apart from the known benefits of SLA, a new paradigm on the synergistic effect of micro- and/or nanotopography and hydrophilicity to enhance wound healing has been proposed in recent

years^{277,348–350}. A surface technology where the acid-etching procedure is similar to that of SLA, performed under N₂ protection and immediately stored in an isotonic saline solution (modSLA and SLActive, Straumann® Institute, Basel, Switzerland), results in significantly increased hydrophilicity¹²⁶. Differences in clinically relevant surface properties modulate the initial biological response during bone healing (Figure 17).

Figure 16. A diagram illustrating different manufacturing methods to obtain hydrophilization of oral implant surfaces. This image was adapted from Rupp et al. (2018)³⁴⁸, ©The Academy of Dental Materials. Published by Elsevier, Ltd (license number: 4521401493137).

Recently, Wennerberg et al. (2013)³⁵¹ validated the spontaneous, time-dependent formation of nanoparticles on SLActive surfaces after being stored in both water and isotonic NaCl solution. The density of nanostructures was shown to be proportional to the level of wettability (Figure 18). Presence of such nanoparticles on a moderately rough topography combined with hydrophilicity increased the interfacial biomechanical fixation after 4 weeks of healing in a model of rabbit tibia, compared to hydrophobic with and without nanostructures²⁴⁷. As aforementioned, SLActive technology ensures a more rapidly surface coverage with blood compared to the ordinary sandblasted acid-etched surface (SAE), enhancing clot formation and adhesion of osteogenic cells^{126,352}.

Several *in vitro* studies support the potential of this surface technology to upregulate expression of various angiogenic factors (*e.g.*, VEGF-A and FGF)^{353–355}, activate the production of anti-

inflammatory factors (*e.g.*, IL-4, IL-10 and TGF- β), and downregulate the expression of pro-inflammatory cytokines (*e.g.*, IL-1 β , IL-6, IL-17, and TNF- α) by osteoblasts^{294,356} and macrophage-like cells^{135,275,357}. SLActive, for instance, has been recently shown to elicit polarization of the adaptive response towards Th2 (wound-healing phenotype), which leads to faster resolution of inflammation and increased stem cell recruitment^{134,292}. As a result, SLActive positively regulates osteogenic differentiation and maturation of MSCs and human osteoblast-like cells^{257,294,358–360}, decreasing osteoclastogenesis and increasing deposition of new bone matrix^{361–363}.

Figure 17. A diagram illustrating different stages of contemporary implant processing from manufacturing to initial biological response. During the period of storage, surface properties might be modified compared to their initial state directly after industrial manufacturing. Chair-side treatments comprise further alterations immediately before implantation. Clinically relevant surface properties modulate the initial host-to-implant interactions. This image was adapted from Rupp et al. (2018)³⁴⁸, ©The Academy of Dental Materials. Published by Elsevier, Ltd (license number: 4521401493137).

Preclinical *in vivo* experimental models^{295,298,312,364–366} and clinical studies have shown that implants with such chemical modification achieve faster and larger amounts of BIC and peri-implant bone density (BD) compared to non-modified implants with otherwise

similar surface topography. In regard to biomechanical performance, higher primary and secondary implant stability quotient (ISQ; %) is recorded following hydrophilic implant placement^{296,365,367}.

Figure 18. Scanning electron micrographs of hydrophilic SLActive surface showing the evolution of nanostructures. (a) Nanostructures are nearly absent immediately after preparation almost. **(b)** After 1 day, a few nanostructures are formed. **(c)** After 3 days, the density of nanostructures increases. **(d)** After 7 days, extra structures are evident. **(e)** After 2 weeks, the evolution of nanostructures has reached its final state. **(f)** The situation after 52 weeks is similar to the latter. These images were reproduced from Wennerberg et al. (2013)³⁵¹, ©John Wiley & Sons, A/S (license number: 4578181128581).

Clinically, chemically modified hydrophilic implants show high ISQ values during early healing time-points³⁶⁸, where otherwise a decrease in h-BIC (experienced as a transient implant stability dip)¹⁷² occurs due to primary interfacial bone remodeling. Nevertheless, the potential of such surface technology to modulate the inflammatory response and increase early bone apposition onto the implant may lessen the healing (non-loading) period and allow more readily immediate or early functional implant loading in patients with reduced bone density³²⁷. Indeed, high long-term survival rates (97% on average) are reported to SLActive following these treatment modalities with similar clinical parameters compared to delayed loading protocols. In general, marginal peri-implant bone loss < 1 mm are reported for such implants over the first 3 years³⁶⁸.

Implant macrodesign

Implant hardware, defined herein as implant macrodesign and related surgical preparation of the osteotomy site³⁶⁹, and the host bone quality/quantity influence the level of primary stability in association with the status of the host bone^{83,370–373}. The implication of interfacial biomechanical properties on the short- to the long-term success rate of oral implants has been extensively emphasized^{374–376}. The definitive purpose of modifying implant hardware is to maximize surface contact area and control microstructural strain generation and distribution at the bone-implant interface, allowing a rapid onset and establishment of osseointegration^{377–379}.

Notwithstanding, surface modifications at the micrometer and nanometer length scale are defined as implant hardware ad-hocs, which modulatory effects on host-to-implant interactions vary according to aspects related to the interplay between implant macrogeometry and surgical technique³⁶⁹. Thus, evaluation of parameters of osseointegration should not be regarded as independent of any of these three key factors³⁷¹.

Primary implant stability and micromotion

By definition, primary implant stability (*i.e.*, initial or mechanical stability as measured by maximum insertion torque values [ITV; N.cm]) is purely a mechanical phenomenon regarded as the ab-

sence of movement at the micrometer range shortly after implant insertion, further distinguished from that obtained due to osseointegration (*i.e.*, secondary/biological stability)^{380,381}.

Clinically, minimum ITV has been recommended as the fundamental criteria for immediate implant loading of single-tooth implants. Ottoni et al.³⁸² previously reported appropriate ITV to be above 32 N.cm in order to avoid implant failure without, however, further correlation to the host bone or implant length. In contrast, Norton et al.³⁸³ recommended a minimum ITV of 25 N.cm as sufficient to achieve medium- to long-term clinical survival (95.5%) and maintain stable marginal bone levels (CBL ranging from 0.1 to 0.5 mm) following insertion into fresh extraction sockets and immediate loading.

Nevertheless, lack of linear relationship between ITV and ISQ or BIC has previously been reported^{383–390}, although experimental studies indicate otherwise^{391–393}. Besides, BIC should rather be interpreted as an indication of the implant osteoconductivity, whereas the absolute implant biomechanical shear strength is determined by a combination of BIC, biomechanical properties of the surrounding bone and, finally, implant design and respective distribution of loading forces³⁹⁴. Despite often conflicting literature data, ISQ remains a key factor to monitor the level of rotation micromotion, ideally suggested to range between 50 and 150 μm ³⁹⁵.

Implant micromovement, as measured by the value of actual micromotion (VAM), is proven to cause a negative cellular response to mechanical stress under functional loading³⁹⁶. VAM has alternatively shown a significant correlation to RTV, BIC, and CBL³⁹⁷ without wound healing impairment solely owing to low ITV^{386,398–400}. Contrariwise, low to nearly absent mechanical interlocking has been demonstrated to accelerate osseointegration and improve the quality of osseointegration in both static and dynamic bone strains^{398,399,401}.

Insertion torque is directly proportional to the degree of radial contact stress and lateral compression applied to the surrounding bone. Peak values translate the level of interfacial resistance to shear stress (*i.e.*, static bone strain or primary stability)^{392,393,402}. Mathematically, ITV is the after-effect resulted from biomechanical

mechanisms of bone cutting and friction occurring during osteotomy and implant insertion as follows³⁸¹:

$$\text{Insertion torque} \approx T_{\text{cutting}} + T_{\text{friction}}$$

Further precisely:

$$T_{\text{cutting}} = C * p * r^2 * k_c$$

$$T_{\text{friction}} = P * A * \mu * r$$

Where:

C: constant strain rate

p: thread pitch

r: radius

k_c : specific cutting force

P: critical pressure on the bone

A ($2\pi r * \text{height}$): surface area in contact with bone

μ : coefficient of friction

Thus, numerous strategies related to implant design have been developed to diminish the level of microstrain ($\mu\epsilon$) concentration in both cortical and trabecular bone at the time of insertion, as well as to improve the implant load-bearing capacity, further discussed in the following sections.

Implant body, length, and diameter

The implant topography at the millimeter length scale describes its macrogeometry, encompassing implant body, length, and diameter, type of abutment connection, as well as thread geometry and its potential for compression and/or cutting²⁵².

Different implant body types have been developed throughout the years. The first modification in the macrodesign was performed on the apex of cylindrical standard Brånemark implant to provide a tapered effect and/or self-tapping of the osteotomy walls, especially in low-density bone^{403,404}. Regarded as the third generation of implants, tapered implants achieve better primary stability compared with cylindrical/parallel-walled implants^{405,406} and are routinely indicated for rehabilitation of challenging cases where thin

cortical and/or poorly density trabecular bone may result in low primary stability (*e.g.*, posterior maxilla, immediate placement in fresh extraction sockets and/or immediate loading of single crowns), revealing higher survival rates^{404,407}. Nevertheless, absence of significantly differences in terms of later failure rates have also been reported^{408–410}.

However, in case of a thin cortical layer, high ITV produced by tapered implants⁴¹¹ may lead to excessive marginal bone resorption, detrimental for achieving osseointegration⁴⁰⁰ (see the section “overcompression and biomechanical strain”). Even though the reduced surface area of tapered implants supposedly increase the stress peak during load transfer⁴¹², no significant differences in terms of parameters of osseointegration (ITV, ISQ and BIC) and MBL were observed after 8 weeks of healing between loaded and non-loaded conditions in a model of minipig mandible⁴¹³. Cylindrical implants, in turn, produce higher stresses and strain energy density in the cancellous bone under immediate functional loading, in this case, compared to tapered implants⁴¹¹.

Implant height and diameter are two main design features that also affect the level of mechanical stability by modifying the total surface area. The fundamental rationale behind the application of longer length implants is based on an increased circumferential area in contact with bone, aiming to create not only greater mechanical interlocking but also long-term resistance to bending moment forces. Even though longer implants (defined in the literature as greater than 8 mm) are shown to better distribute loading stress generated from occlusal forces, they do not have any impact in decreasing the degree of friction or compression generated at the interfacial region. Furthermore, implants longer than 12 mm do not offer any further benefit in terms of transmitting loading forces to the surrounding bone⁴¹⁴.

In turn, shorter-length implants are manufactured for rehabilitation of sites limited by the height of residual alveolar ridge but presenting adequate bone volume (*i.e.*, posterior region of the mandible and maxilla), preventing possible morbidity associated with augmentation procedures⁴¹⁵. However, they present lower predictability and variable survival rates⁴¹⁶. Particularly when inserted in poorly dense bone, shorter implants lead to low total stability dur-

ing the early period of healing, increasing the risk of failure compared to longer implants with otherwise the same diameter²¹⁷. In contrast, few studies observed that implant length does not appear to affect implant survival^{417–419}.

As briefly mentioned, ITV is a function of implant diameter in both bone cutting and friction, raised to the power of 2 in the former situation. Wider implants (> 4 mm) were developed to overcome limitations regarding implant macrodesign (*i.e.*, cylindrical body)⁴²⁰, insufficient bone height⁴²¹, or immediate placement in fresh extraction sockets in posterior regions of the jaw^{422,423} that are commonly associated with decreased initial stability and, consequently, more failure events.

Several studies have shown that wider implants produce higher ITV due to the increased surface contact area and result in high long-term survival rate (97.76%)⁴²⁴ compared to both standard- (> 3.5 mm) and narrow-diameter implants. Stress concentration in cortical bone seems to decrease by increasing implant diameter, whereas superior stress distribution in trabecular bone is observed around longer implants^{425,426}. Extra-narrow implants (< 3.0 mm) show significantly lower implant survival than standard-diameter implants and are mainly indicated for the rehabilitation of highly atrophic alveolar ridges⁴²⁷. Yet, short implants presenting wide diameter (≥ 4.0 mm) reveal similar survival rates compared to longer implants with standard and extra-narrow implants^{428–430} due to corresponding biomechanical performance. Although implant diameter does not seem to affect MBL, the crown-to-implant ratio is suggested to not exceed 1.7 in narrow implants in order to prevent early CBL as a consequence of excessive stress concentration at the cortical bone⁴³¹.

Thread-shape designs

Thread geometry has an impact on primary stability³⁷⁰ and interfacial stress concentration, more effectively of the immediately loaded than the osseointegrated implants⁴³², and thereby may influence the amount/rate of new bone formation³⁷⁹. In this regard, functional geometrical elements encompass thread shape, width, depth, pitch, face and helix angle that provide adequate interfacial mechanical resistance against rotational movement and overloading

(Figure 19). From a theoretical standpoint, while thread shape, width, depth, pitch, and the associated potential for either cutting or compression might contribute more significantly to primary stability and stress generation, thread shape, face and helix angle might have a greater effect on stress distribution and strain orientation as a result of applied forces.

Figure 19. A graphic illustration of the threads' geometric profile (shape, width, depth, pitch, and face angle) and implant macrodesign features (helix angle, inner and outer implant diameter, and lead). Double-threaded implants have a lead that is double the pitch⁴⁴³. The original images of the implants were provided by Neodent®, Curitiba, PR, Brazil.

The most commonly applied thread shapes include square, trapezoidal, buttress, reverse buttress, and triangular (standard V-thread) design (Figure 20). Accordingly, various implant body designs and thread geometry, in interplay with surgical technique

(*e.g.*, final drill diameter), will generate different ITV due to distinct levels of cutting and compressive stress concentration upon implant insertion, influencing stress peak^{370,433,434}. Square and trapezoidal cutting chambers yield greater primary stability^{372,435}, whilst triangular threads result in lower ITV³⁸⁶, whether integrated or not with cutting flutes in the apical portion of the implant. In turn, compressive threads are better suggested to benefit implant insertion in low-density bone^{436,437}.

Figure 20. A graphic illustration of different implant thread designs. This image was adapted from Abuhussein et al. (2010)⁴⁴³, ©John Wiley & Sons, A/S (license number: 4522520672891).

Accordingly, Nevins et al.⁴³⁸ reported that self-cutting threads reduce stress peak and dislocate bone chips residues into the osteotomy site, resulting in lesser ITV but faster and greater BIC in the initial healing period in a preclinical model of low- to moderate-density bone (2 and 4 weeks). In spite of higher primary stability, compressive threads caused extensive bone remodeling and delayed bone response during the same period of evaluation. However, differences in ISQ and MBL were not detected between implant

groups, but rather a slight decrease in value in compressed bone sites after 7 days of healing.

Likewise, the stress generation and distribution patterns in osseointegrated implants under occlusal load (100 – 200 N) differ whether in cortical or trabecular bone, but may be similar amongst different thread designs^{91,439}. Due to its significant inelastic deformation, microstructural stress is highly concentrated around the implant neck (18.3 MPa)⁴³⁹, more specifically at the first implant thread, both during implant placement and under functional loading^{432,439,440}. In the cancellous bone, the maximum concentration stress is located around the implant apex (13.3 MPa).

Several *in vitro* studies applying computational models, such as finite element analysis (FEA), have shown that trapezoidal⁴⁴¹ and triangular thread shaped-design⁸³ yield lower biomechanical stresses to the surrounding cortical and trabecular bone, respectively, in comparison with square threads. Specifically, trapezoidal threads are shown to provide minimum stress concentration and greater interfacial shear strength under functional loading³⁷².

Eraslan et al.⁴³⁹ found no differences in the level of stress distribution with varying the thread design, but instead in the intensity of stress in both cortical and trabecular bone. While reverse buttress generated the largest compressive area, equally followed by buttress and square threads, triangular threads produced the minimum intensity area. In the cortex, in particular, Mosavar et al.⁴⁴⁰ verified that square-shaped threads result in the most favorable levels of pressure and shear stresses compared to triangular, buttress and reverse buttress.

Subsequently, thread pitch comprises the distance from the middle of one thread to the next thread and has consistently shown to impact the dynamics of osseointegration at the initial stage of healing⁴⁴². Commonly, implant systems include single-threaded design, which presents an equal pitch and lead. Recently, some manufacturers have introduced double- and triple-threaded implants presenting a lead that is double or triple the size of the pitch, respectively, where threads run parallel one to the other, facilitating implant insertion⁴⁴³. For example, a double-threaded implant presenting a small pitch of 0.7 will be inserted 1.4 mm in each complete spin. However, by increasing the number of parallel threads, the

implant helix angle is also increased, facilitating implant insertion⁴⁴⁴. This might be beneficial to minimize stress due to reduced friction and lateral compression but not to generate adequate primary stability that supports immediate and early loading forces as the level of micromotion is relatively worsened.

Thus, single-threaded implants possessing larger threads with small pitch might be more favorable in terms of mechanical fixation in challenging cases where low primary stability may be encountered^{401,433,445,446}. However, Chowdhary et al.⁴⁴⁷ showed that pitch shortened threads do not offer superior initial or secondary stability compared to threads with large pitch³⁷³, but rather lead to higher bone density in poor-quality bone due to increased bone compression, as observed in a model of rabbit femur.

Another aspect that influences the level of static and dynamic bone strain is related to implant collar design (*i.e.*, implant crestal module). Particularly, micro-threads affects stress distribution and supports bone formation in cortical bone compared to implants in the absence thereof⁴⁴⁸. Specifically, triangular-shaped micro-threads contribute to maintaining MBL in nonfunctional immediately loaded implants⁴⁴⁹. Evidently, there exists a limited body of literature evaluating mechanical properties of varying thread geometry during surgical placement and their related effect on bone healing patterns which, combined with great variability in methodological assessment, do not allow a pertinent scientific synthesis and further clinical translation.

Surgical preparation of the implant site: the drilling technique

Allegedly, the main aim of a surgical protocol is to obtain adequate implant primary stability to bear immediate or early functional implant loading¹⁷³. Surgical instrumentation is based on the concept of bone drilling, a well-known osteotomy preparation technique that applies drills to cut and extract and/or laterally condense the bone in order to create a cylindrical or tapered defect site⁴⁵⁰.

During drilling, the kinetic energy produced by rotation and motion of the drill is converted into thermal energy that causes a progressive increase in intraosseous temperature as a result of frictional forces and shear stresses^{451,452}. The degree of heating is depend-

ent on drill design (body and shank design, and cutting efficiency [helical or straight flutes and drill wear])⁴⁵³, rotational or cutting speed (rotation per minute; rpm) and feed rate (relative to the motion of the drill against the workpiece; mm/min), axial thrust force (N)^{454,455}, and cooling system (double irrigation vs. internal or external irrigation)⁴⁵⁶. Another potential factor contributing to more heat generation during instrumentation is the thickness of the cortical bone due to its greater stiffness and thermal conductivity compared to the trabecular bone^{457,458}. The density of the latter also determines the variation in heat conduction.

As indicated before, the type of the host bone not only influences the level of mechanical shear strength but also determines cell activity and dynamics of new matrix deposition towards the achievement of biological stability^{266,459,460}. If a temperature higher than 47°C is generated over 1 min, heat-induced osteonecrosis may occur inhibiting tissue repair and the bone regenerative potential⁴⁶¹.

Hence, researchers have primarily investigated a variety of modifications in the surgical protocol to avoid overheating of the bone walls, mainly by attempting to minimize trauma from instrumentation²⁵. Over the years, different drilling techniques have been implemented in order to control the cutting efficiency and lateral compression applied to different types of bone and, finally, to modulate the host response to implants and accelerate osseointegration.

Osseodensification vs. conventional implant subtractive drilling

In order to generate minimal trauma during drilling, Albrektsson et al.²⁵ proposed the use of a conventional subtractive method that employs stepwise instrumentation at a positive rake angle. This drilling technique includes a series of multi-fluted drills that gradually cut the bone and remove debris with the fleeting of each flute as the osteotomy dimension is enlarged and which instrumentation sequence is contingent upon the host bone and implant design. In order to reduce operation time and risk of infection, preclinical studies have only recently analyzed the effect of simplified subtractive protocols by means of reducing the number of drills in the instrumentation sequence. Remarkably, this modification in the con-

ventional drilling technique shows comparable bone response during the early stages of osseointegration^{462–464}.

The association between implant macrogeometry, more specifically body shape and thread design, and osteotomy dimension usually result in a dimensional mismatch between the outer implant diameter and the surgically instrumented bone wall. This mismatch is often described in the literature as the implant-last drill discrepancy (IDD, mm and %), defined as the relative dimensional mismatch between the outer implant diameter and the bone wall in the implantation bed⁴⁶⁵. Depending on the interplay between implant design and related surgical protocol (final drill diameter and cutting potential), different degrees of frictional forces and compressive stress will be generated resulting in high or low static strain, translated into ITV^{386,466}. Therefore, the hardware design has the ability to direct the type of bone healing mode.

Previously, the use of osteotomes in sites presenting low-density bone was assumed to enhance peri-implant bone density and secondary implant stability owing to condensation of trabecular bone⁴⁶⁷, a phenomenon that is currently highly contradictory⁴⁶⁸. Wang et al.⁴⁶⁹ demonstrated at a maxillary posterior alveolar bone model of mice that lateral bone condensation (IDD = 20%) accumulates higher crestal strain (approximately 80%), which leads to microfractures, extensive marginal bone resorption, and limited biomechanical properties compared to the conventional subtractive drilling.

More recently, literature has emerged that favors a non-subtractive drilling concept, termed osseodensification, to increase primary stability in poorly dense bone through compression of the trabeculae in the osteotomy site^{470,471}. Unlike the regular subtractive drilling, tapered, multi-fluted burs containing a cutting chisel and tapered shank are applied at a negative rake angle during osseodensification, controlling the level of bone compression as the drill progresses inwards the osteotomy site. A layer of highly dense compact bone is then created at the peri-implant interface and, upon implant insertion, a press-fit situation is generated according to the manufacturer's recommendations. This technique was initially postulated based on the fact that compressed cancellous bone has a spring-back effect due to its elastic modulus capable of providing

greater static strain⁴⁷². Remaining non-vital bone debris function as nucleating sites of compacted autografts, further improving the osteoconductivity in the osteotomy site, bridging newly formed bone to the implant surface⁴⁷³.

Several preclinical studies have evaluated the potential of this surgical procedure (IDD $\approx$ 0.4 mm/15%) to support osseointegration in both models of small and large animals. In general, greater ITV and RTV, and lesser VAM are reported for osseodensification in comparison with regular drilling after a healing period of 4 and 8 weeks in an animal model of low-density bone⁴⁷⁴. By increasing bone density during osteotomy, the bone response appears to be positively modulated with no signs of healing impairment subsequent to nucleation of compact autografts, resulting in higher BIC values in comparison with subtractive regular-drilling technique.

Interestingly, these outcomes befall irrespective of the implant body design (tapered vs. cylindrical) and surface technology (machined vs. acid-etched) as observed after 3, 6, and 12 weeks *in vivo* in the iliac crest of sheep^{475–478}. Additionally, no differences in the levels of ISQ or temperature generation were demonstrated among different drilling techniques⁴⁷². Yet, the evidence to date does not include studies reporting on clinical outcomes for osseodensification drilling compared to other subtractive drilling techniques, which remains to be investigated.

Contemporary, aiming to attain adequate primary stability by applying conventional implant subtractive drilling, manufacturers recommend a final drill that commonly generates a dimensional mismatch as per standard, creating a press-fit phenomenon⁴⁷⁹. A series of recent studies evaluating standard drilling techniques report the IDD ranging from 0.2 to 0.5 mm (3 - 15%)^{401,465,466,480–483}. The theoretical background to this concept is that tightly fit scenarios, where the dimension of the osteotomy walls matches the inner implant diameter while the threads engage within the bone, allow increased surface contact area⁴⁷⁹.

Previous retrieval studies analyzing human specimens have shown that implants fitting tightly into the bone attain high bone microstructural organization under functional loading due to maturation and continuous remodeling^{484–486}. The bone surrounding these implants, which are mostly inserted in the mandible, present-

ed a compact and mature LB with osteons in close proximity to the interface at both cortical and trabecular regions⁴⁸⁷.

In the pursuit of relatively higher primary stability levels, specifically in cases of immediate loading where thin cortical and/or poorly dense trabecular bone is encountered (*i.e.*, atrophic posterior maxilla), immediate implant placement, and shorter implants, lateral compression of the surrounding bone is performed by means of smaller-sized drills by employing an undersized subtractive drilling technique^{488–490}. Under-dimensioned implant sites are characterized by a mismatch of the inner and outer implant diameters in relation to the osteotomy wall, which is substantially narrower ($IDD > 0.5 \text{ mm}/15\%$)^{170,465,491} (Figure 21a).

Figure 21. A graphic illustration of the osteotomy dimension relative to the implant's external diameter and microstrain generation by different ITV. (a) Undersized preparation of the implant site where the dimension of the osteotomy walls matches the inner implant diameter while the threads engage within the bone (press-fit situation; high ITV). **(b)** Oversized preparation with a nearly similar dimension between the bone defect and implant outer diameter (low ITV). **(c)** Oversized preparation with a gap (*) between the bone defect and the implant outer diameter (no torque). This gap has been demonstrated to be filled with fibrous tissue if excessive micromotion occurs. **(d)** High ITV result in increased frictional and compressive forces around the threads and the implant body. **(e)** Photo-elastic stress generated by high ITV. **(f)** Small region of moderate compressive strain with low ITV where only 0.015-0.020 mm of the thread engaged within the bone. b, bone. f, fibrous tissue. i, implant. ITV, insertion torque values. These images were adapted from Insua et al. (2017)⁵²⁴, ©Wiley Periodicals, Inc (license number: 4522571287662).

As a result, significantly higher ITV and h-BIC are created in poor-quality bone in comparison with the standard drilling technique^{371,387,481,482,492}. Although surface topographical modifications at the micrometer length scale contribute to primary stability due to increased friction during implant placement, its osteoconductivity might be diminished due to changes in surface roughness regardless of the implant thread geometry⁴⁹³.

Nevertheless, due to lack of guidelines on the exact amount of IDD on the basis of a given clinical situation, surgeons frequently rely on the manual perception of primary stability, generalizing increased ITV as a predictive value to achieve successful osseointegration without considering the mechanical properties of distinctive types of bone.

As far as thick cortical and/or highly dense trabecular bone is concerned, the level of static strain created at the surrounding bone following standard and undersized drilling techniques defines the extent of tissue damage. It has been previously shown that excessive compression of the host bone can impair osseointegration in the early stage of healing when an IDD greater than 15% is applied⁴⁶⁵, eventually leading to increased CBL in the first year of loading⁴⁹⁴.

Moreover, the effect of undersized drilling on primary stability is limited with increased thickness of the cortical layer (above 2 mm)⁴⁹⁵, whereas different levels of narrow drill diameters do not offer any major benefit but only generate high stress rates^{384,483,496} (Figure 21d, e). When frictional force and compressive stress beyond the physiological microstrain limits of the peri-implant bone is produced⁴⁹⁷, microfractures will be created^{498,499} (Figure 22a), triggering different patterns of inflammatory response and bone remodeling at the early period of osseointegration, which allegedly results in bone necrosis as shown in different *in vivo* animal models^{384,401,465,483,500–502}. The biological and mechanical basis of this event is further discussed in the following section.

Osteocompression and biomechanical strain

The crestal-cortical bone thickness and density, and cancellous bone density are the most influential aspects on the level of strain generated when subjected to compressive stress than the drilling

technique^{503,504}. Particularly, thick poorly vascularized cortical bone is concerned to be at higher risk of overcompression as less porous, non-elastic, and poorly vascularized bone is more inclined towards microstructural stresses due to a microstrain threshold of 935 and 3.264 $\mu\epsilon$ in the presence of high- and low-density cancellous bone, respectively, as analyzed in the posterior mandible⁵⁰⁴.

When a thick crestal bone presents a relatively low density, the maximum strain significantly increases in the model of a poorly dense trabeculae (6.451 $\mu\epsilon$) in comparison with the opposite situation (1.214 $\mu\epsilon$). On the other hand, a thin cortical layer presents a greater variation in the values of microstrain among low- (11.270 – 13.050 $\mu\epsilon$) and high-density cancellous bone (2.546 – 2.683 $\mu\epsilon$), irrespective of the crestal bone density.

Compressive stress applied to compact bone, despite its high resistance to fracture, originates long microcracks in a cross-hatched fashion⁵⁰⁵, while a rather arc-shaped microcracking pattern, following the long axis of osteons, is formed under shear stress⁵⁰⁶. Likewise, mechanisms of crack deflection at higher strain rates are associated with bone stiffness. Lamellar structure of cortical bone presenting a lower density has a central role to preserve tissue integrity by deflecting microcracks inside the osteons boundaries^{499,507}. In turn, the minority of larger cracks occurring in high-density cortical bone are restricted to the cement line, which increases tissue damage⁵⁰⁶ and is normally associated with collagen deformation and delamination (*i.e.*, bone tear) of LB at the primary thread engagement area⁴⁸².

Following instrumentation and implant insertion, immediate vascular interruption and bone overcompression impair the microcirculation⁵⁰⁸. Accordingly, lack of blood supply associated with mechanosensitive cell-mediated response to microdamage lead to ischemia and osteocyte necrosis, characterized by empty lacunae or containing osteocyte debris at the tissue level, only evident after 24 h^{110,509}. Overall cellular activity and bone metabolism are further disturbed by damaging the osteocytic interconnecting system (OLCS)⁵¹⁰.

Subsequently, compression necrosis (*i.e.*, non-viable bony sequestrs) progresses in association with non-solving inflammation, due to the synergetic effect of bacteria colonization⁵¹¹, eliciting tar-

geted remodeling of mineralized tissue between the first threads^{384,483,496,500}. This process is termed as “necrosis dieback” and consists of multiple coalescent bone remodeling sites (BMUs) occurring after 1 week following implant placement⁵⁰⁰.

As a consequence, excessive bone resorption/remodeling will occur following the first weeks upon implant placement with low appositional rate (2 – 5 $\mu\text{m}/\text{day}$; Figure 22b). In spite of some studies showing greater RTV in undersized sites, a substantial decrease of interfacial shear strength occurs between 2 and 4 weeks *in vivo*^{379,466,482}, while the opposite occurs in sites receiving standard instrumentation. Normally after 6 weeks of healing, no significant differences are found between standard and undersized drilling protocols, either in terms of histomorphometrical parameters of osseointegration or biomechanical properties.

Figure 22. Optical micrographs of implants placed in undersized sites surgically instrumented to the inner implant diameter in a model of Beagle dog. (a) Press-fit situation depicting mechanical stability ensured by h-BIC after 2 weeks *in vivo*. Red arrows, microcracks. Dark blue arrow, bone remodeling. **(b)** Regions of injured bone between the dashed line and the implant were substantially resorbed after 4 weeks *in vivo*. Green arrow, BMUs at the extension of a microcrack. BMUs, basic multicellular units. h-BIC, host bone-to-implant contact. These images were adapted from Coelho & Jimbo (2014)³⁶⁹, ©Elsevier, Inc (license number: 4535940304135).

According to histological descriptions, BMUs are observed along bone debris and microcracks, especially in the cortical bone⁵⁰¹, depicted as void spaces that are partially filled with newly formed

bone, reestablishing BIC. This process clearly characterizes the interfacial remodeling healing mode. Comparable amounts of BIC between surgical protocols were reported in several studies^{384,399,466,500,512,513}, despite a minor inconsistency in the literature possibly due to differences in animal model and implant system. While some authors have shown an increased bone response in under-dimensioned sites compared to standard preparation of the implant site^{482,502,514}, a series of recent studies prove otherwise^{465,511,515}.

The extensive resorptive component of the tissue repair process leads to loss of structural integrity, where marginal bone loss is a common finding during early peri-implant wound healing in experimental studies^{170,465,511,516}. Severe bone resorption is shown to decrease the regenerative potential of the tissue, directly affecting the establishment and maintenance of osseointegration^{170,515}. Theoretically, increased bone remodeling in bone type IV may remarkably increase micromotion with eventual osseointegration delay and/or impairment under immediate functional loading due to reduced bone strain distribution⁵¹⁷. However, clinical data is insufficient to allow further comparisons.

Regarding conventional loading protocols, observational studies have not shown any significant differences in terms of MBL and survival rates of implants inserted with either standard protocols or undersized drilling techniques (IDD > 20%) in the posterior maxilla^{480,491}. In cases of thick cortical layer and/or highly dense trabeculae, surgical preparation of the cortex is further enlarged with profiling (*i.e.*, countersink) in addition to tapping. Undersized drilling with no additional profiling causes deleterious effects on MBL and osseointegration, particularly around immediately loaded implants⁵¹⁵, which may lead to early failure⁵¹⁸.

Oversized preparation of the implant site

Recently, a compressive strain release technique has been investigated by employing a larger final drill outer diameter, often related to as oversized drilling, through a stepwise drilling instrumentation to achieve either sites with similar dimension^{384,401,502} or over dimension between the bone defect and the implant outer diameter (IDD = 0 mm or ≤ -1 mm, respectively)^{399,466,500} (Figure 21b-c).

Implant insertion in over-dimensioned sites result in low ITV (< 20 N.cm) compared to undersized sites (Figure 21f), particularly in low-density trabecular bone^{384,401}.

The implant thread design, more specifically pitch, shape, and depth, in interplay with the osteotomy dimension create closed healing chambers, namely void spaces, shaped in the inner thread region between the inner implant diameter and the surgically instrumented bone wall^{152,171,519,520}. Healing chambers allow immediate blood clot stabilization following implant insertion, thereby creating an osteoprotective microenvironment which early healing kinetics are capable of positively modulating cell-mediated responses towards intramembranous-like healing pathway¹⁵².

As thoroughly described before (see the section “wound healing phenomena”), the provisional fibrin matrix acts as a scaffold enabling cellular migration of osteogenic precursors to the implant surface, where the development of a highly osteogenic tissue occurs through contact osteogenesis and bone nucleation deprived of primary interfacial bone remodeling^{378,379,401}. Spatial bone filling of healing chambers happens in a multidimensional manner throughout its volume: on the implant surface and from the surgically instrumented pristine walls towards the central region of the chamber³⁷⁹ (Figure 23a).

Marin et al.⁵²⁰ investigated the differential impact of various healing chamber configurations on the early healing in a model of dog mandible. It was shown that thread shape and length significantly affect BIC: trapezoidal- and buttress-shaped threads promoted the highest values, followed by power threads (two square-shaped threads with varying size and shape within the single lead) and, finally, triangular threads. Even though healing chamber dimensions did not affect bone density, it significantly increased over time up between 3 and 5 weeks. Owing to this type of healing mode, the newly formed bone is replaced by LB containing multiple primary osteonic structures, rich in osteocytic lacunae directly in contact and in close proximity with the implant surface, observed after 5 weeks of healing (Figure 23b).

A retrieval study evaluating progressive mechanical adaptation to functional loading in humans showed that bone remodeling in healing chambers advance to a multidirectional Haversian-like

channel system, which reveals increased hardness and elastic modulus at the long term, regardless of the bone type³⁷⁶. Thus, the mechanically competent bone formed in over-dimensioned sites substantially differs in relation to the poorly vascularized compact bone created in narrower sites⁴⁸⁷. However, both healing pathways lead to temporal bone microstructural development towards a more organized morphology.

Figure 23. Representative micrographs of implants placed in over-dimensioned sites in an *in vivo* model of Beagle dog. (a) At 3 weeks, multidirectional WB filling of the healing chamber throughout its volume. **(b)** At 5 weeks, the newly formed WB is replaced by LB containing multiple primary osteonic structures. **(c)** Intramembranous-like healing in over-dimensioned sites allows contact osteogenesis which process of BOM deposition by OBs results in multiple L in the MBF directly in contact and in close proximity with the implant surface. BOM, bone organic matrix. CB, cortical bone plate. L, lacunae. LB, lamellar bone. MBF, mineralizing bone front. OBs, osteoblasts. WB, woven bone. These images were adapted from Coelho & Jimbo (2014)³⁶⁹, ©Elsevier, Inc (license number: 4535940304135).

In spite of limited theoretical background on the effects of healing chambers on osseointegration, the majority of the preclinical studies show that oversized drilling accelerates and enhances %BIC

and, therefore, secondary stability, regardless of the implant macrodesign. In contrast, there are conflicting results in the literature with respect to the interfacial mechanical properties of this technique to exceed that obtained by implants placed with higher ITV, hypothetically due to lower bone compression and densification^{466,483}. The available literature on this topic is based on studies on animals, with varying bone metabolism and bone type, in addition to a diversity of implant designs, and lacks a hierarchical database to drive future research efforts and, finally, clinical decision making.

Clinically, Norton⁵²¹ has shown in a cohort prospective study that self-tapping implants compressing only the apical portion of both healed and fresh implantation sites yielded low ITV (5 - 20 N.cm), resulting in moderate primary stability (67%, on average) with a substantial increase to approximately 80% in the first 3 months. After 1-year follow-up, the success rate was 100% and no CBL was detected in 71.4% of the cases receiving either immediate or late loading, while only 21.4% of them presented less than 0.5 mm of marginal bone resorption.

Nevertheless, it is evident that high ITV does not necessarily increase the early mechanical performance of implants, but rather causes a provisional decrease in implant stability. Therefore, strategies to reduce bone compression meanwhile providing sufficient primary stability have been investigated.

The hybrid healing pathway: bringing together interfacial remodeling and intramembranous-like bone healing mode

Overall, investigations on the effect of the dimensional interplay between implant macrogeometry and surgical instrumentation provide insight on how implant hardware design modulates the bone response towards either interfacial remodeling or intramembranous ossification, changing the progression of osseointegration⁴⁰¹. The ultimate goal, however, is to achieve an adequate and stable primary stability in parallel to a rapid onset of osseointegration with no extensive resorption of damaged bone by osteoclasts.

Subsequently, some studies have demonstrated the benefit of the hybrid healing pathway to enhance osseointegration and load-bearing capacity of implants placed in over-dimensioned sites,

which integrates thread regions engaged within the bone in the presence of healing chambers. This can be seen in the case of experimental implants possessing power threads, specifically engineered for insertion in over-dimensioned sites to allow partial engagement of the larger thread tip in association with small healing chambers⁵²².

Another case consists of tapered implants presenting a dynamic progressive thread profile with various shapes and dimensions through the implant length⁵¹⁶. Such implant design, in tandem interplay with oversized preparation of the site, including profiling and tapping, may prevent compressive strain in highly dense crestal and trabecular bone, while providing adequate mechanical resistance to micromotion. The rationale behind this association lies upon the fact that the tip of the buttress and triangular self-tapping threads slightly engage into the bone surrounding the implant apex, while square and trapezoidal threads form healing chambers in the central coronal portion between the implant bulk device and the site walls. So far, this concept has only been applied by a few authors who have demonstrated the benefit of the hybrid healing pathway to simultaneously enhance the mechanical stability and accelerate osseointegration as demonstrated in preclinical studies^{501,523}.

In this scenario, a transient decrease in implant stability that follows events of interfacial remodeling of damaged bone or sites initially in direct contact to the implant surface may be immediately compensated by a rapid WB filling of healing chambers in the early healing period (Figure 24). In this sense, employing a minimally traumatic surgical instrumentation technique is an important aspect to be considered in order to prevent osteotomy line dieback caused by thermal or compression necrosis (see the section “surgical preparation of the implant site: the drilling technique”), particularly in highly dense trabecular bone and thick marginal layer⁵²⁴. This allows normal bone metabolism and a stable balance between healing modes. As aforesaid, peri-implant bone healing is synergistically modulated by implant hardware ad-hocs, such as topographical and chemical surface properties, solely in the presence of a bone strain within the physiological limit.

Figure 24. A representative micrograph of an implant placed in an over-dimensioned site presenting hybrid healing. Observe a partial engagement of the thread tips in association with healing chambers due to a mismatch between implant thread outer diameter and surgical instrumentation outer diameter (blue line). In tandem, a transient decrease in implant stability that follows events of interfacial remodeling of damaged bone or sites initially in direct contact to the implant surface (red arrows) may be immediately compensated by a rapid woven bone filling of healing chambers in the early healing period (yellow arrows) from all available surfaces, including the instrumented bone after its dieback due to surgical instrumentation (green line). This image was reproduced from Coelho & Jimbo (2014)³⁶⁹, ©Elsevier, Inc (license number: 4535940304135).

DISSERTATION'S RATIONALE

Despite the excellent performance of most major implant systems, much research on the biological response to hierarchical aspects of new implant technologies for enhancing BIC and their biomechanical properties is propagated by established or new implant manufacturers. Evaluation of the effect of modifications in implant design and surface topography and/or chemistry composition on the bone response in *in vivo* preclinical models using small and large animal platforms is mandatory prior to clinical testing, also for regulatory reasons⁵²⁵. Particularly, a novel hydrophilic moderately rough Ti surface ($\theta < 5^\circ$) has been suggested to play a critical role during the early healing period and establishment of successful osseointegration in a preclinical *in vivo* study using the rabbit tibia model. However, its histomorphometrical parameters of osseointegration and biomechanical properties in a large animal platform is yet undetermined.

Furthermore, several studies have recently shown that surface modifications with increased hydrophilicity do not only regulate the innate immune response but can also polarize the adaptive immune response towards either pro-inflammatory or wound-healing phenotypes. Recently, a study has shown that implant surfaces lacking macrophage populations compromise stem cells adhesion and promotion of an anti-inflammatory and regenerative microenvironment¹³⁴.

Even though biomaterials are shown to influence the activation status of APCs, immunomodulatory factors that control cell polarization and differentiation from the monocyte/macrophage lineage (*e.g.*, MNGCs and OCs) in response to implant surface properties

remain uncertain and increasingly contradictory^{132,135,526}. Specifically, there is limited knowledge regarding the possible combined modulatory impact of implant macrogeometry and surface hydrophilicity on osteoclastogenesis.

In addition, the optimal level of primary stability, to lessen the healing period and enhance osseointegration, has remarkably been questioned as it does not depict the quality of the osteotomy itself, the degree of bone compression and microdamage³⁸⁴. In addition, this raises questions in relation to excessive removal of bone through oversized instrumentation and its effects on osseointegration and interfacial shear strength when compared to standard and undersized drilling techniques. There is limited knowledge regarding the optimal dimensions of oversized preparation of the implant site for the various types of implant designs, in terms of faster osseointegration and biological stability.

Despite evidence supporting osseointegration of implants installed in over-dimensioned sites in preclinical animal models, available data must be reviewed in a systematic manner in order to avoid misinterpretation due to innumerable experimental designs. Moreover, studies investigating the effect of oversized drilling and the hybrid healing pathway on parameters of osseointegration and MBL in thick, highly dense cortical bone are limited and warrant further investigation.

AIMS

*A theory is a supposition which we hope to be true,
a hypothesis is a supposition which we expect to be useful;
fictions belong to the realm of art; if made to intrude elsewhere,
they become either make-believes or mistakes*

George Johnstone Stoney (1826 - 1911), Irish physicist

The general aim of the present thesis was to evaluate the possible impact of aspects of current oral implant technology, *i.e.*, surface hydrophilicity, implant macrogeometry, and surgical preparation of the implant recipient site, on implant osseointegration.

More specifically, it was aimed:

1. To evaluate the histomorphometrical parameters (mineralized bone-to-implant contact [BIC] and relative peri-implant bone density [BD]) at implants with a sandblasted, dual acid-etching, with or without an additional chemical modification (SAE-HD and SAE, respectively) at early stages of osseointegration in the tibia of Beagle dogs (study I).
2. To evaluate the biomechanical properties (removal torque value [RTV] and a complete set of intrinsic properties [removal energy and connection stiffness]) at SAE-HD and SAE implants at the early stages of osseointegration in the tibia of Beagle dogs (study II).

3. To evaluate the influence of the implant thread design (trapezoidal vs. triangular threads) and surface hydrophilicity (SAE-HD vs. SAE) on osteoclast (OC) differentiation, activation, and survival *in vitro* in a model of RAW 264.7 cells (study III).
4. To systematically assess the current evidence on the effects of oversized surgical preparation of the implant site in terms of biomechanical properties and histomorphometrical parameters of osseointegration in experimental animal models and to perform a meta-analysis of the estimate of efficacy of oversized vs. standard or undersized surgical preparation of the implant site (study IV).
5. To evaluate the effect of oversized surgical preparation of the implant site on marginal peri-implant bone level (MBL) and histomorphometrical parameters (BIC and BD) at tapered implants with a progressive thread geometry following 12 weeks *in vivo* in the mandible of minipigs (study V).

MATERIALS AND METHODS

*Progress is made by trial and failure; the failures are generally a
hundred times more numerous than the successes;
yet they are usually left unchronicled*

Sir William Ramsay (1852 - 1916), Scottish chemist

Experimental units and study design

Titanium implants

CpTi grade IV implants (study I, II, and V) were manufactured by Neodent® (Curitiba, Paraná, Brazil) for preclinical *in vivo* analyses. In study I and II, a total of 48 implants (10 mm x 4.0 mm, L x Ø) presenting similar macrodesign with a cone-morse implant-abutment connection interface, cylindrical body, and double triangular threads (V-shaped) with high potential for bone compression (TMX, marketed as Titamax® CM EX), was used (Figure 25a).

In study V, 10 implants (11.5 mm x 3.5 mm, L x Ø) presenting a full dual tapered body with a hybrid contour, an active apex, and helicoidal flutes (HLX, marketed as Helix®) were analyzed (Figure 25b). This implant presents a dynamic progressive thread geometry with double threads varying between compressing square and trapezoidal on the coronal area, reverse buttress on the central aspect, and self-tapping triangular threads (V-shaped) on the apical part of the implant with varied dimensions finishing in 0.55 mm. Due to

its potential for either bone compression or cutting, HLX is indicated for all types of bone and immediate implant placement post-extraction. HLX presents platform switching and the Grand Morse® (GM) implant-abutment connection interface characterized by a deep 16° morse taper connection.

All experimental units were initially sterilized by gamma (γ)-irradiation according to standard procedures from the International Organization for Standardization (ISO 11137, 2013) prior to processing, stored in sealed glass tubes to prevent hydrocarbon contamination, and shipped under sterile conditions.

Figure 25. Representative illustrations of the macrodesign features and threads' geometric profile (shape, width, depth, pitch [mm], and face angle) of the experimental implants. (a) Titamax® CM EX (study I and II). (b) Helix® Grand Morse® (study V). The images of the implants were provided by Neodent®, Curitiba, PR, Brazil.

Titanium disks

In study III, CpTi grade IV disks (12 mm x 4 mm, Ø x L) exhibiting 2 different macrodesign (TMX and HLX [prototype]) were also manufactured by Neodent® for preclinical *in vitro* analyses. Differences regarded thread shape, width, depth, pitch, face angle, and the potential for either bone compression and cutting. TMX presents double triangular threads with similar grooves of 0.37 mm (Figure 26a). HLX presents a dynamic progressive thread geometry

varying between square, trapezoidal and triangular design exhibiting grooves of varied dimensions finishing in 0.55 mm (Figure 26b).

Surface topographical and physico-chemical properties

Both implants and disks present a moderately rough surface (Figure 27) produced by means of sandblasting with 1.4 - 1.8 μm abrasive particles and dual acid-etching with a combination of HCl and H₂SO₄ (SAE, marketed as NeoPoros®; study I, II, and III). After SAE, chemically modified implants received in addition proprietary technology treatment, including microwave treatment under nitrogen protection to prevent air exposure, and insertion in 0.9% isotonic saline (NaCl) solution, resulting in significant hydrophilicity (SAE-HD, marketed as Acqua™; study I, II [prototype], III, and V). The 3D surface roughness parameters of NeoPoros® and Acqua™, investigated by means of CLSM³¹², are described in Table 1.

Figure 26. Graphic illustrations and scanning electron micrographs of the thread geometric profile (shape, width, depth, pitch, face length [mm] and helix angle) of the experimental disks (study III). (a) Titamax (TMX). (b) Helix (HLX).

The chemical composition of the different surfaces, as examined by XPS, revealed the atomic percentage values of carbon (49 and 17) and oxygen (22 and 59) in SAE and SAE-HD, respectively. The surface energy and wettability were investigated by means of a static contact angle (θ) analysis by means of the sessile drop technique applying simulated body fluid solution. SAE surfaces reveal a hydrophobic behavior ($\theta > 90^\circ$), whereas SAE-HD surfaces display hydrophilic properties ($\theta < 5^\circ$).

Preclinical *in vitro* analyses

In study III, *in vitro* analyses were performed to evaluate the influence of the implant thread design and surface hydrophilicity on osteoclast differentiation, activation, and survival.

Figure 27. Scanning electron micrographs of the surface topography of the experimental units, hydrophobic SAE (NeoPoros®; study I, II, and III) and hydrophilic SAE-HD (Acqua™; study I, II, III, and V) presenting a moderate micro-roughness. (a) Overview at x10. (b) High magnification at x7000.

TABLE 1 Three-dimensional roughness parameters of a sandblasted, dual acid-etched surface (hydrophobic SAE; NeoPoros®) and with an additional chemical modification (hydrophilic SAE-HD; Acqua™)³¹²

Parameter	SAE	SAE-HD
S _a (µm)	1.44 ± 0.15	1.26 ± 0.17
S _{sk}	-0.51	-0.43
S _z (µm)	14.57 ± 1.00	16.20 ± 7.8
S _{ds} (1/mm ²)	658.67 ± 27.42	643.33 ± 37.74
S _{dr} (%)	1.51	1.2

S_a: average height amplitude; S_{sk}: amplitude distribution curve; S_z: average distance between the highest peak and the deepest valley; S_{ds}: number of peaks per area unit; S_{dr}: mean slope and the developed area ratio.

Mouse monocyte/macrophage cell line (RAW264.7)

RAW 264.7 gamma nitric oxide deficient (NO-), an immortalized macrophage-like cell line derived from adult male BALB/c mice by the Abelson murine leukemia virus, were obtained from American Type Culture Collection (ATCC® TIB-71™, LGC Standards GmbH, Wesel, Germany). These cells have been extensively applied in *in vitro* studies due to their expression of high levels of RANK and capacity to differentiate into bone-resorbing OCs (RAW-OCs) by treatment with RANKL⁵²⁷ (Figure 28). They are considered superior over the use of primary cells, such as bone-marrow derived macrophages (BMMs), because of their purity, sensitivity to differentiation, prompt maturation, and close correlation in gene expression and signaling^{528,529}.

Figure 28. Fluorescence-based staining of TRAP, F-actin, and DAPI of RAW-OCs. (a, d) Small cells with the average 3-5 nucleus. (b, e) Large cells with average nuclei number > 10. (c, f) Box magnified demonstrating multiple nuclei (red and white arrows). Color code: purple, TRAP; red, actin cytoskeleton; blue, cell nuclei. DAPI, dihydrochloride nucleic acid stain. F-actin, actin filaments. RAW-OC, macrophages treated for differentiation to osteoclasts. TRAP, Tartrate-Resistant Acid Phosphatase. These images were adapted from Kong et al. (2019)⁵²⁹ and they are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0).

Cell expansion, seeding, and differentiation to osteoclasts on titanium disks

RAW 264.7 cells adhere to tissue culture-grade plastic through cation-dependent integrin receptors and other cation-independent receptors. Prior to study commencement, cells were cultured at a density of 2×10^6 cells/ml in 75 cm² filtered tissue culture flasks (Sarstedt AG & Co, Nümbrecht, Germany; Figure 29a) in Dulbecco's Modified Eagle Medium (DMEM) high glucose containing L-glutamine and phenol red supplemented with 10% heat-inactivated fetal bovine serum (FBS) and Penicillin-Streptomycin 10.000 µg/ml (Gibco®, Gaithersburg, MD, USA), at appropriate atmospheric conditions (37°C, 5% CO₂ and 95% relative humidity) in a Forma™ Steri-Cycle™ incubator (Thermo Fisher Scientific®, Waltham, MA, USA).

Cells were sub-passaged every 3 to 4 days by mechanically harvesting with a cell scraper for no more than 10 passages while routinely checking for their health status under phase-contrast microscopy (Olympus® CK30™, Tokyo, Japan). The cellular confluency was determined by using the trypan blue exclusion method on a Neubauer hemocytometer. At the end of each passage, cells were about 90% confluent (Figure 29b-c).

In order to differentiate RAW264.7 cells to OCs (RAW-OC formation), cells were seeded on the experimental disks placed in a 24-well plate (Figure 29d) at an initial population density of 3×10^5 /ml/well and subsequently treated with 100 ng/ml RANKL (PeproTech®, Rocky Hill, NJ, USA) and cultured for 5 days. In all experiments, cells were allowed to achieve confluency for 12 hours before RANKL-induced differentiation. RANKL-containing medium was replaced after 3 days. The medium was removed after 5 days and cells were processed for further analysis.

RAW-OCs differentiated on polystyrene, *i.e.*, cells seeded directly on polystyrene-surface wells and cultured for 5 days in the presence of RANKL, were used as positive control [CCPC (+); Figure 29e-f]. Cells cultured in the same fashion for only 12 h were applied as negative control [CCPC (-)]. The assay positive control group, applied in the cell viability and gene expression assays, comprised cells treated with RANKL for 4 days and, additionally, stimulated with 100 ng/ml LPS for 24 h (CCPC-LPS) to secrete in-

flammatory cytokines. RAW264.7 cells are extremely sensitive to LPS endotoxin from gram-negative bacteria.

Figure 29. Images and optical micrographs of cell expansion, seeding, and differentiation to osteoclasts on polystyrene-surface wells and titanium disks. (a) RAW 264.7 cells were initially cultured for expansion for 3 days until they reached 90% of confluency, as observed on **(b, c)** optical micrographs. **(d)** To differentiate RAW 264.7 to OCs (RAW-OC formation), cells were seeded on 3 experimental disks/group and treated with 100 ng/ml of RANKL and cultured for 5 days, as depicted on **(e, f)** optical micrographs. OCs, osteoclasts. RANKL, receptor activator of nuclear factor kappa B ligand. Overview at x10 and high magnification at x40.

Thus, 4 experimental groups and 3 control groups were tested ($n = 3$), in triplicate:

1. CCPC (-): negative control.
2. CCPC (+): positive control.
3. HLX/SAE-HD: progressive thread and hydrophilic surface.
4. HLX/SAE: progressive thread and hydrophobic surface.

5. TMX/SAE-HD: triangular threads and hydrophilic surface.
6. TMX/SAE: triangular threads and hydrophobic surface.
7. CCPC-LPS: assay positive control.

Osteoclast differentiation and viability

Specific enzymatic Tartrate-Resistant Acid Phosphatase (TRAP) activity

As previously mentioned, acid phosphatase (AP) dephosphorylates phosphate groups from phosphate esters under acid conditions, producing inorganic phosphates and organic radicals (see the subsection “cellular components of bone tissue - osteoclasts”). Specifically, increased TRAP (type 5b AP) synthesis indicates RAW-OC formation through Mφ fusion.

The activity of this metalloproteinase was measured using the Acid Phosphatase Colorimetric Assay Kit (Abcam®, Cambridge, UK), according to the manufacturer’s instructions. The kit uses *p*-nitrophenyl phosphate (*p*NPP) as a phosphatase substrate, which turns yellow when dephosphorylated by AP. Readout of optical density at 405 nm was performed with a spectrophotometer. The *p*NP standard curve was plotted to identify the *p*NPP concentration and then TRAP activity was calculated as follows:

$$\text{TRAP activity (mU)} = A/(V/T) * D$$

Where:

A: amount of *p*NP in the sample well (μmol)

V: sample volume added into the reaction well

T: reaction time

D: sample dilution factor

Conclusively, one unit of AP is the amount of enzyme causing the hydrolysis of one micromole of *p*NPP to *p*NP per min (mU). The final concentration of TRAP was normalized to cell viability.

3-(4,5-Dimethylthiazol-2-yl) -2,5-Diphenyltetrazolium Bromide (MTT) colorimetric assay

Cell viability of RAW-OC were assessed after 5 days of culture using the Vybrant® MTT Cell Proliferation Colorimetric Assay Kit

(Thermo Fisher Scientific®), according to the manufacturer's instructions.

This method determines the cell viability via mitochondrial metabolic activity based on the reductive cleavage of the water-soluble MMT, a yellow salt, to a dark blue insoluble compound (formazan) by mitochondrial dehydrogenase of living cells (*i.e.*, transfer of electrons from one molecule to another by the action of oxidoreductases [dehydrogenases])⁵³⁰. The formazan is then solubilized, and the absorbance levels of each well read at 570 nm using a spectrophotometer, which color intensity correlates to the number of viable cells.

Gene expression analysis: reverse transcriptase real-time quantitative polymerase chain reaction (RT-qPCR)

In study III, gene expression related to osteoclastogenesis, OC activity and survival were analyzed after 5 days of differentiation by means of a deoxyribonucleic acid (DNA) amplification technology, a two-step RT-qPCR.

Nucleic acids are one of the four essential macromolecules of eukaryotic organisms, alongside with proteins, lipids, and complex carbohydrates (polysaccharides), responsible for cell growth, function, and replication⁵³¹. DNA (double strand) and ribonucleic acid (RNA, single strand) are polymeric molecules composed units called nucleotides, which are made of three different components: a 5-carbon sugar (deoxyribose and ribose, respectively), a phosphate group, and a nucleobase (a nitrogen-containing monomeric base). DNA strands are folded onto a helix and held together due to hydrogen bonding of nucleotides, *e.g.*, (deoxy)nucleotide triphosphates, (d)NTPs. The number of carbon atoms in each end of the sugar backbone, specifies the type of prime end as three and five (3'- and 5'-end, respectively), where there is a free hydroxyl group⁵³¹.

DNA and RNA not only differ in their structure but also on function and stability⁵³². Genomic DNA forms a chromosome and contains the genetic information implicated in coding, regulation, and expression of genes. A gene is a specific sequence of nucleosides in DNA (three letter codons) comprised of noncoding and coding regions (introns and exons, respectively). The latter, in a

single strand of DNA, acts as a code to generate messenger RNA (mRNA; *i.e.*, transcription) through the action of RNA polymerase, directing the synthesis of specific functional proteins in the cytoplasm (*e.g.*, growth factors, enzyme, etc.; *i.e.*, translation).

More specifically, following signal transduction and transcription (see the section “wound-healing phenomena: cell-to-cell communication and healing pathways”), the expression profile of target genes is represented by pre-mRNA. These primary transcripts of mRNA are not stable and are further processed through splicing (elimination of introns to form a contiguous coding sequence). In addition, 5' capping (addition of a 7-methylguanosine cap to protect the mRNA from degradation) and polyadenylation (addition of a polynucleotide chain consisting of adenosine monophosphates [poly(A) tail] to the 3'-end) contribute to stabilizing the mRNA. Finally, steady-state mRNA is transported through nuclear pore complexes to cytoplasm where translation by the ribosome and the action of ribosomal and transfer RNA molecules (rRNA and tRNA, respectively) result in synthesis of proteins, orchestrating the cellular response to multiple stimuli¹²².

One important aspect to be considered is that gene expression is regulated by the level of transcription and mRNA stability, translational rate, and protein turnover (*i.e.*, the balance between protein synthesis and degradation), in addition to other biological factors, such as cell cycle and its maturational stage^{533,534}. Therefore, interpretation of data must be performed in view of the type of cell population.

Total RNA extraction and purification

Considering that gene coding for a specific protein is transcribed into mRNA and further translated, isolation of this macromolecule through cellular disruption is necessary to enable gene expression analysis. PCR amplifies complementary DNA (cDNA) products reverse transcribed from mRNA; this process is used by retrovirus to integrate into the host's genome through cloning. Therefore, single isolation of genomic DNA (gDNA) does not offer information about which genes are actively being expressed at a specific time-point, in addition to adding molecules originated from both dead and living cells. Further purification of mRNA is performed to ex-

clude inhibitory substances (*e.g.*, genomic DNA and other impurities) that may interrupt the coding sequence of the protein or interfere with the PCR reaction.

Because mRNA is quickly degraded by ribonucleases (RNases; enzymes present in the surrounding environment)⁵³⁵, microbiological and sterile techniques must be taken to avoid RNase contamination, and the samples must constantly be maintained under proper refrigeration during all procedures. Furthermore, pre-PCR processing strategies, such as sampling preparation, RNA extraction method, and chemistry of reverse transcription (RT) and PCR, were performed to assure 100% efficiency amplification, which means that there is an exact doubling of your template every cycle.

Total RNA (mRNA and microRNA) extraction and purification were performed applying the RNeasy Mini Kit (Qiagen®, Hilden, Germany) that consists of different chemical and physical techniques. The final concentration of RNA, a measure of how strongly the gene is expressed, is influenced by the amount and type of sampling. Thus, after 5 days of culture, a starting material of 1×10^7 cells was obtained as recommended by the manufacturer by mechanically harvesting and centrifuging the samples at 300x g for 5 min while keeping them refrigerated in a 24-well plate. The supernatant was removed and cells were disrupted by adding 350 μ l of a lysis buffer (RLT). The cell lysate was vortexed for 1 min to ensure complete homogenization and carefully transferred to new 1.5 ml RNase-free tubes and diluted with 350 μ l of alcohol 70%.

The samples were purified with spin column membranes and collection tubes. Up to 700 μ l of the sample was transferred to a RNeasy spin column placed in 2-ml collection tube followed by centrifugation at $\geq 8.000x$ g for 15 s. The samples were washed with different RNA wash buffers (RW1 and RPE) and a deoxyribonuclease (DNase) solution to exclude the genomic DNA. Each washing step was followed by centrifugation at $\geq 8.000x$ g for 15 s, and the flow-through was carefully discarded. Finally, the final centrifugation at $\geq 8.000x$ g for 2 min was performed to wash the membrane. The spin column was then placed in a 1.5 ml collection tube and the RNA was eluted with 30 μ l of nuclease-free water, added directly to the spin column membrane and centrifuged at $\geq 8.000x$ g for 1 min. Total RNA fraction was quantified with a

NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific®) and further normalized to yield an equivalent concentration among different samples.

Reverse transcription

In order to quantify the mRNA gene expression levels with PCR, it first needs to be converted into cDNA by means of reverse transcription⁵³⁶. This reaction was performed using the high capacity iScript™ cDNA Synthesis Kit (Bio-Rad®, Hercules, CA, USA), which included a reverse transcriptase-buffer (MgCl₂) system containing RNase inhibitor, a single-stranded sequence of oligonucleotide deoxythymine [oligo(dT)], and random hexamer primers mixed with nuclease-free water and the RNA template (50 ng/μl). The complete mix was incubated in a thermal cycler (CFX Connect 96™ Real-Time System) and the reaction was run and monitored with CFX Manager 3.0 software (Bio-Rad®), according to the manufacturer's instructions. Subsequently, the cDNA served as template for amplification and relative quantification of the selected genes of interest by means of the real-time qPCR.

Gene expression analysis

Introduced in 1980 by Kary Mullis⁵³⁷, PCR is a powerful method in molecular biology for detection, identification, and quantification of gene expression through the generation of thousands to millions of copies of a target DNA sequence (*i.e.*, amplicons or product), in the 3'-5'-end direction⁵³⁷. DNA amplification is achieved by using designed forward and reverse primers that are complementary to this region under specific thermal cycling conditions⁵³⁸. The conventional PCR technique applies agarose gel analyses after completing the reaction, which results in increased detection time and residue contamination.

Real-time PCR, also called quantitative (qPCR), was developed in the 1990s and allows to monitor amplification in real-time by measuring the kinetics of the reaction in the exponential phase where the concentration is doubled in each cycle⁵³⁹. Detection of the accumulating PCR product in real-time is done with a fluorescent reporter molecule (*e.g.*, a non-specific DNA-binding dye or a sequence-specific probe), excluding the necessity for further gel

analysis. Increasing of fluorescence emitted from the fluorophore is translated into cumulative target amplicons.

TaqMan is a target-specific, dual-labeled hydrolysis (oligo) probe, named after the thermophilic bacterium *Thermus aquaticus*⁵⁴⁰, that presents a fluorophore/reporter (5'-end) and a quencher (3'-end). The latter suppresses the fluorescence emitted by the reporter, inhibiting any signal while they are next to each other⁵⁴¹.

The PCR reaction consists of three sequences with varying temperature repeated for many cycles (~39; Figure 30). In the first series, a process called denaturation, the two strands of cDNA are physically separated into single strand molecules at a high primer melting temperature (T_m , 95°C). Secondly, as the temperature is lowered (60°C), primers and the probe bind to single stranded complementary target sequences during annealing. The two DNA strands become templates for DNA polymerase to selectively amplify the complementary strand downstream of the PCR primers, a process termed as elongation. When extension reaches the hydrolysis probe, the 5'-3' exonuclease activity of the heat-resistant enzyme *Taq* polymerase cleaves the reporter and quencher creating an increase in fluorescence relative to the concentration of the target double-stranded amplicons⁵⁴². At the end of the reaction, thousands to millions of copies of a particular DNA sequence are generated⁵³⁸.

TaqMan® Gene Expression Assays (Applied Biosystems®, Foster City, CA, USA) designed for mouse species were used. The probe is labeled with a 6-carboxyfluorescein (FAM) dye and a quencher that attaches to shallow grooves in the DNA helix (3'-minor groove binder [3'-MGB]), which offers 100% efficiency amplification, higher sensitivity and specificity compared to a non-specific DNA-binding dye assay⁵⁴³. The chemistry of the latter, in addition to non-specificity to any particular DNA sequence which requires primer designing, results in the release of the dye during denaturation, drastically reducing fluorescence and sensitivity of the assay if not operating well-developed protocols⁵⁴⁴.

Figure 30. A graphic illustration of the PCR reaction using a TaqMan hydrolysis probe. It consists of three sequences with varying temperature repeated for approximately 39 cycles which generate thousands to millions of copies of a particular DNA sequence. This image was adapted from Bass et al. (2010)⁵⁴² and it is licensed under the Creative Commons Attribution License (CC BY).

The reaction mix (5 µl of SsoAdvance2d™ Universal Probes Supermix; Bio-Rad®) included a thermally stable DNA polymerase-buffer system containing target sequence-specific primers, deoxynucleotide triphosphate (dNTPs), enhancers, stabilizers, and a blend of passive reference dyes mixed with 3.5 µl of nuclease-free water, 0.5 µl of TaqMan®, and 1 µl of the gene transcript (cDNA template). The complete reaction mix was incubated in the thermal cycler and the qPCR reaction was run and monitored according to the protocol.

The gene expression levels of TRAP1, Cathepsin K (CTSK), Matrix Metalloproteinase (MMP)-9, Calcitonin Receptor (CALCR), Arginase 1 (ARG1), Interleukin (IL)-6, Tumor Necrosis Factor (TNF)-α, and Bcl-2-associated X protein (BAX) were investigated. All the reactions were run in 96-well plates. Two technical replicates of each sample were tested for each gene assay and negative template controls (purified PCR-grade water) were included in all PCR reactions to check for possible reagent contamination, according to the MIQE guidelines⁵⁴⁵. Melting curves were obtained after the amplification to exclude the presence of primer-dimer formation (products specificity).

The two most commonly used methods to analyze qPCR reactions are absolute and relative quantification. The former determines the total number of amplicons by applying standard curves and comparing a test sample to a control. The relative gene expression data analysis applies the $2^{-\Delta\Delta C_t}$ method⁵⁴⁶, which is based on the normalization of cycle values (C_t) of the target gene sequence to C_t of the reference genes (ΔC_t) – which expression is considered to be relatively stable for the type of cells – and again to a positive control sample ($\Delta\Delta C_t$), as follows:

$$2^{-\Delta\Delta C_t} \\ 2^{\Delta C_t - C_t \text{ control}}$$

Where:

2: 100% PCR amplification efficiency

ΔC_t : C_t target gene – C_t reference gene

This method was applied by normalizing C_t of target genes, related to osteoclastogenesis, to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and beta-actin (ACTB) C_t ⁵⁴⁷, and to C_t of the positive control [CCPC (+)]. Usually, more than 1 reference gene is used to test their reliability in a given sample⁵⁴⁸. All mathematical calculations were performed in the Excel Software (Microsoft®, Portland, OR, USA).

In order to correct interplate variations, an inter-run calibration sample (Interplate Calibrator FAM Probe, TATAA Biocenter AB, Göteborg, Sweden) was used in triplicate in every run, according to the manufacturer's instructions and integrated into the qPCR data analysis workflow⁵⁴⁵.

Qualitative and quantitative image analyses of osteoclast proliferation, adhesion, and morphology

Scanning electron microscopy (SEM) analysis

SEM analysis of the experimental groups was applied to qualitatively assess cell proliferation, adhesion, and morphology, *e.g.*, filopodia projections (slender cytoplasmic projections present in migrating cells). This microscopic method uses a high energy beam of electrons to create images of the specimen, which is sputtered with a thin layer of a conductive metal⁵⁴⁹. During microscopy imaging, a beam of electrons is focused onto the sample, which interaction produces excited secondary electrons, back-scattered electrons, and x-ray photon energy. Finally, a detector collects the emitted particles to produce the image of the sample surface.

In study III, following 5 days of culture on the Ti disks, cells were fixed in Sørensen phosphate-buffered glutaraldehyde solution (4%, 0.1 M, pH 7.4) overnight. The samples were rinsed twice in phosphate buffered saline (PBS, 0.1 M, pH 7.4) and dehydrated in a graded ethanol series. After critical point drying (BAL-TEC CPD 030, BalTec Group, Pfäffikon, Switzerland), the samples were sputter-coated with gold/palladium in a sputter coater at 1.2 kV, 15 mA, 0.02 mbar for 20 seconds (Polaron SC7640, Quorum Technologies Ltd, Kent, UK).

Images at different magnifications (10x, 100x, 500x, 1500x and 3000x) were obtained under a SEM microscope (JSM-5600LV, JEOL®, Peabody, MA, USA) at 5 kV and the working distance of 6

mm and 30 mm. The analysis was performed in an area of interest (AOI) equivalent to the thread pitch of each macrogeometry (grooves of 0.37 mm).

Confocal laser scanning microscopy (CLSM) analysis

OC proliferation, adhesion, and morphological structures on the experimental groups and CCPC (+) were qualitatively and quantitatively investigated on cells immunoassayed for actin filaments (F-actin, cytoskeleton) and DNA (cell nuclei), and imaged under CLSM. Immunocytochemical staining and fluorescence imaging are the most valuable techniques available in biomedical research to analyze the anatomy and function of molecules on cell samples, such as protein and antigens, by means of a secondary antibody that has a conjugated fluorophore⁵⁵⁰.

Fluorescence is a natural phenomenon produced by light interaction with a matter, in this case, a microscopic specimen which produces a certain degree of diffraction⁵⁵¹. Under fluorescence microscopy imaging, this process is better represented by the transmission of light through a pinhole, where the light diffusion forms an Airy disk. The size of this disk depends on the numerical aperture (NA) of the objective lens, which represents the range of angles over which the system can receive or emit light – the larger the NA, the lesser is the diffraction (narrower Airy disks), resulting in high-resolution fluorescent images.

In CLSM, a focused beam of light is projected on a single point of the specimen through the objective, which fluorescence image is brought to focus in front of the detector by adding a pinhole in a conjugate focal plane in close proximity to a dichroic mirror⁵⁵². The transmission image is then captured by another detector, meaning that the confocal microscope has two coincidental focal points, which reject information from outside the plane of focus, reducing background fluorescence (noise).

In study III, following 5 days of differentiation, cells were fixated and permeabilized (Image-iT™, Invitrogen®, Carlsbad, CA, USA). The actin cytoskeleton was stained with phalloidin staining solution (Alexa Fluor™ 488, Invitrogen®), which contains a peptide, phalloxin, that binds to both small and large filaments, at a final concentration of 0.1 µM/well mixed with 1% bovine serum albu-

min (BSA) to reduce nonspecific background staining, and incubated for 20 minutes at 24°C. The cell nuclei were stained with 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI nucleic acid stain, FluoroPure™, Invitrogen®) at a concentration of 300 nm/well for 15 minutes.

Samples were rinsed several times in PBS and allowed to air-dry. Subsequently, disks were fixed in a small plexi containing a 12-mm customized perforation, mounted in a permanent mounting media (ProLong™ Gold Antifade Mountant, Invitrogen®) and covered with cover glasses for microscopy (Menzel-Gläser, 24 x 24 mm, no. 1.5, VWR International, Radnor, PA, USA).

Confocal microscope images (TCS SP8 DLS™, Leica®, Wetzlar, Germany) were obtained with optical section separation (z-stack) of an AOI equivalent to the thread pitch of each macrogeometry (grooves of 0.37 mm), comprising a length and voxel depth of 0.60 x 0.60 x 0.37 mm (X x Y x Z; Figure 31) at 25x magnification (oil immersion). Images were processed and used for 3D assessment of osteoclast size (µm²).

Figure 31. Graphic illustrations of the AOI under CLSM. (a-c) HLX disks. (d-f) TMX disks. (a, d) The AOI included the thread pitch of each macrogeometry (grooves of 0.37 mm; blue area within the trapezoidal square). (b, e) Three-dimensional view. (c, f) Cross-section of AOI: length and voxel depth were 0.60 x 0.60 x 0.37 mm (X x Y x Z). AOI, area of interest. CLSM, confocal laser scanning microscopy.

63x magnification (water immersion) micrographs were used for measurements of actin ring size (µm) and analysis of cell prolifera-

tion and adhesion by analyzing morphological structures (*e.g.*, filopodia projections and podosomes; the latter are actin-derived structures associated with integrin-mediated cell^{115,116,553}). Images were analyzed using ImageJ 2.0 software (National Institutes of Health, Bethesda, MD, USA).

Systematic review and meta-analysis

Systematic review of interventions involves a methodical and reproducible search strategy applicable to a wide range of databases to maximize identification of primary study designs (observational clinical studies⁵⁵⁴, *e.g.*, prospective randomized or non-randomized clinical controlled trials, and preclinical *in vitro* and *in vivo* studies⁵⁵⁵) that are eligible to answer a well-defined research question that is formulated based on ‘PICO’ (population, intervention, comparison, outcome)⁵⁵⁶. This question determines the inclusion and exclusion criteria to assess the eligibility of the studies.

By limiting the inclusion criteria, systematic review reduces selection bias and increases homogeneity among studies, allowing a more reliable evaluation of treatment effectiveness (estimates of effects) during meta-analysis, compared to conventional narrative review⁵⁵⁷. In addition, it aims to analytically address the methodological quality and internal validity of selected studies, based on risk of bias (RoB) tools and different guidelines that intend to improve the reporting of research and are consistent with the emphasis of the review⁵⁵⁸.

Specifically to preclinical studies in experimental animal models, results extracted from those presenting potential biases and low methodological quality (*e.g.*, unbalanced baseline characteristics for the intervention and control groups; non-randomized and non-concealed allocation sequence and outcome assessment [selection bias]; non-blinded investigators and outcome assessors to interventions and treatment groups [detection bias]; non-standardized housing and husbandry, and post-operative care; etc.) should be interpreted with precaution⁵⁵⁵.

Study IV follows the guidelines of the Preferred Reporting of Systematic Review and Meta-analysis (PRISMA) statement, an evidence-based minimum set of methodological aspects to be reported, which aimed to systematically assess the current evidence on

the effects of oversized surgical preparation of the implant site on osseointegration in experimental animal models⁵⁵⁹. Before commencement of the present systematic search, a comprehensive review protocol aiming to reduce selection bias was established and approved by all authors^{560–562}.

PICO question and inclusion criteria

The focused ‘PICO’ question was: in preclinical *in vivo* and *ex vivo* experimental models, do oral implants inserted in overdimensioned sites present greater biomechanical properties and histomorphometrical parameters of osseointegration compared to implants inserted in standard- and/or undersized-drilled implant sites?

Studies reporting original research comparing oversized implant drilling technique to standard/or undersized implant drilling technique were included in the present systematic review and meta-analysis according to the following criteria described in Table 2. No restrictions were applied regarding animal species, year of publication, sample size, implant system, surface technology, time of implant placement, and observation period.

TABLE 2 Inclusion and exclusion criteria for the systematic review

Inclusion criteria	Exclusion criteria
Randomized or non-randomized controlled preclinical <i>in vivo</i> studies	<i>Ex vivo</i> and/or <i>in vitro</i> studies
Use of intra-oral or extra-oral experimental sites of skeletally small or large animals	Studies comparing different implant macrogeometries in the test and control groups
Presence of at least one control group (<i>i.e.</i> , standard and/or undersized drilling technique)	Studies evaluating oversized drilling applied only in the cortical layer (<i>i.e.</i> , profiling)
Studies published in English until July 2018	Studies reporting orthodontic implants (mini-screw implants), short implants (≤ 6 mm) and/or orthopedic implants
Including one of the following analysis: micro-computed tomography (μ CT), radiographic assessment, histomorphometric and histologic analysis, or biomechanical test	Studies evaluating regenerative techniques and immediate placement of implants using autogenous bone and/or biomaterial-based grafting in combination with barrier membranes

Search strategy, screening methods, and study selection

Electronic databases of MEDLINE (via PubMed), Web of Knowledge, Scopus, and Cochrane Wiley Library were searched for relevant English-language literature with a platform-specific search strategy up to and including July 2018. The search strategy consisted of combinations of medical subject headings (MeSH) terms, keywords enclosed in both title and abstract for all databases, and Boolean operators, including: animal models and dental implants (population); oversized and non-undersized drilling technique (intervention); undersized and standard drilling technique (comparison); insertion torque, implant stability quotient, bone-to-implant contact, bone density, removal torque, and marginal bone level (outcomes).

A double-blind three-stage screening process was carried out by two independent reviewers (R.N.R.J. and A.S.) to assess relevant studies. At the first and second stages, screening of titles and abstracts of the search-identified studies were performed, respectively. Studies showing to assemble the eligibility criteria, or those presenting insufficient information qualified for inclusion, were selected for further assessment. The final selection of articles at the third stage screening-process was provided by proof of reading of full texts of all eligible studies. Following each stage, a consensus was taken by R.N.R.J. and A.S. to discuss agreements and disagreements about inclusion or exclusion criteria of each study. In case of disagreement, a third co-author was designated as the mediator (D.Z.B.). Agreement between reviewers was described by interrater reliability (Cohen's kappa coefficient; κ)⁵⁶³.

Data extraction

One reviewer (R.N.R.J.) was entitled to extract the data of the studies in the final selection, followed by an appraisal by a second reviewer (A.S.). Descriptive summaries of respective biomechanical properties and histomorphometrical parameters of osseointegration were further entered into tables, including the authors' conclusions. Standard errors of means and confidence intervals (CI), when reported, were converted to standard deviation values. In case of missing or misplaced data, the corresponding author was contacted via email. Mean $\pm$ standard deviation (s.d.) were finally reported.

Significant differences between groups were accordingly identified by symbols.

The implant-last drill discrepancy (IDD, mm) was calculated to classify control and experimental groups for meta-analyses and further comparisons, according to Table 3 and Figure 32.

TABLE 3 Control and test groups according to the drilling technique

Group	Drilling technique	IDD (mm; %)
Control	Press-fit/standard drilling technique to undersized drilling technique	≥ 0.2 mm; ≥ 3%
Test OD	Oversized drilling technique to achieve either nearly or exactly similar dimension between the bone wall and the implant outer diameter (stress-free)	0.0 – 0.1 mm; 0 – 2%
Test GAP	Oversized drilling technique presenting a gap between the bone wall and the implant outer diameter (friction-free)	≤ –0.1 mm; ≤ –3%

IDD: implant-last drill discrepancy.

Due to the large variation in the methodology of the eligible studies, studies were pooled based on homogeneity regarding ‘PICO’. Thus, the dimension of oversized implant sites was classified into two levels, according to the most frequently reported IDD’s in the pertinent literature.

Quantitative biomechanical (ITV and RTV; N.cm) and histomorphometric data (BIC and BD; %) were then extracted for meta-analysis. When studies included multiple standard and/or undersized drilling protocols, the one with the least IDD was included in the control group. Outcomes reported in different time-points were pooled in three major periods of healing as reported in the literature as short- (1 – 2 weeks), intermediate- (3 – 4 weeks) and long-term healing (≥ 5 weeks).

Risk of bias and methodological quality assessment

The SYRCLE’s (Systematic Review Centre for Laboratory Animal Experimentation) Risk of Bias (RoB) tool for animal studies, based on the Cochrane Collaboration RoB tool, was applied to assess and report the internal validity of the included articles⁵⁶⁴. Risk of bias was assessed as ‘high’ (–), ‘unclear’ (?), or ‘low’ (+).

Figure 32. Graphic illustrations of different drilling techniques. (a) Undersized drilling (UN) depicting the mismatch of the inner and outer implant diameters in relation to the implantation bed wall. (b) Standard drilling (press-fit; SD) where tapping of the host site is performed with the implant while the threads engage within the bone. Oversized surgical preparation of the implant site depicting healing chambers formed due to the interplay between macrogeometry and surgical technique: (c) nearly similar dimension between the implant outer diameter and the bone wall (test OD), and (d) the presence of a gap between the implant and parent bone wall (test GAP). Red dotted lines: outer implant diameter. Transparent blue, last drill dimension over-imposed on the implant. IDD: implant-last drill discrepancy (mm). These images were adapted from Neodent®, Curitiba, PR, Brazil.

TABLE 4 Summary of the *in vivo* experimental design of studies I, II, and V

Study	Animal model, age, weight, and limb	Implant design L x Ø	Control	Test	Time <i>in vivo</i> (n/group/period)	Outcomes of measurement
I	6 male Beagle dogs 18 m, 13 - 15 kg Tibia	Titamax® CM EX 10 x 4.0 mm	SAE	SAE-HD	2 and 4 w (n = 6)	Histomorphometrical: BIC and BD Biomechanical: RTV, connection stiffness, and removal energy
II						
V	5 female minipigs 24 m, 50 - 60 kg Mandible	Helix GM® Acqua™ 11.5 x 3.5 mm	SD	OD	12 w (n = 5)	Histomorphometrical: BIC, BD, and MBL Biomechanical: ITV

BD: bone density (%); BIC: bone-to-implant contact (%); connection stiffness (N.cm/rad); ITV: insertion torque values (N.cm); m: months; MBL: bone-to-implant contact (mm); OD: oversized drilling technique; removal energy (x10⁻²J); removal torque values (N.cm); SAE: sandblasted, dual acid-etched surface (hydrophobic); SAE-HD: sandblasted, dual acid-etched surface with additional chemical treatment (hydrophilic); SD: standard drilling technique; w: weeks.

Modified ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines were applied to assess and report the quality of the studies regarding relevant items⁵²⁵.

Preclinical *in vivo* analysis

Experimental animal models and study design

Preclinical *in vivo* experiments were performed in extra-oral (study I and II) and intra-oral (study V) models of large animals, Beagle dogs and mixed breed minipigs, respectively. Sample size was calculated based on information in previous studies using the same animal models to allow evaluation of possible differences in bone-to-implant contact between groups. Details on the experimental design of each study are described in Table 4.

In study I and II, *in vivo* analyses were performed to evaluate the histomorphometrical parameters and biomechanical properties at SAE and SAE-HD sur-

faces at early stages of osseointegration. The Beagle dogs were kept in the animal care facility of Federal University of Uberlândia (Brazil) and received hard-pellet food twice per day.

In study V, *in vivo* analyses aimed to evaluate the effect of an oversized implant site on MBL and parameters of osseointegration. The minipigs were kept in the animal care facility of Aristotle University of Thessaloniki (Greece) and received restricted soft commercial food to control their weight gain.

All animals were acclimatized in the animal care facility for 2 weeks previously to the surgical procedures with *ad libitum* access to water, under relative humidity and 12-hour circadian rhythm. The level of pain, distress, or suffering was daily assessed during the observation period to ensure the welfare of the animals under a rigorous health monitoring program.

The dogs were randomly pair-housed in standard shelters (1 x 1.5 m kennel) at the ambient temperature of 22°C. The minipigs were group-housed in standard boxes (4 mm²) with long straw as bedding material to satisfy rooting behavior at the temperature-controlled environment of 18°C.

Ethical approvals

The usage of animal models requires the implementation of strict guidelines comprising ethical standards, study design and performance, and reporting of the results. In study I and II, the animal experimental protocol was approved by the Bioethics Committee for Animal Experimentation (CEUA, protocol no. 098/10) at the Federal University of Uberlândia, Brazil, and followed the normative guidelines of the National Council for Animal Control and Experimentation (CONCEA).

Study V was approved by the General Directorate of Rural Economy and Veterinary Medicine, Department of Animal Health and Veterinary Welfare, Drugs and Applications (protocol no. 449328-2583), Region of Central Macedonia, Thessaloniki, Greece. Throughout the trials, handling and intervention of the animals followed the Federation for Laboratory Animal Science Associations (FELASA) guidelines (Directive 2010/63/EU).

All preclinical *in vivo* studies are reported according to the ARRIVE guidelines, in regard with relevant items⁵²⁵.

Surgical procedures

Anesthesia protocol

Surgical procedures were performed under general anesthesia and aseptic conditions. In study I and II, sedation of the Beagle dogs was achieved with an intramuscular (i.m.) injection of atropine sulfate (0.044 mg/kg; Faramagícola S.A., São Paulo, SP, Brazil) and xylazine chlorate (8 mg/kg; Virbac do Brasil Indústria e Comércio Ltda., São Paulo, SP, Brazil). Then, general anesthesia was induced with 15 mg/kg ketamine chlorate (Faramagícola S.A.). Intra- and peri-operative infection control was achieved with a prophylactic i.m. injection of Pentabiotic (30.000 IU/kg; Pentabiótico Veterinário® 1.200.000 IU, Fort Dodge Saúde Animal Ltda., Campinas, SP, Brazil). Each proximal tibia was initially shaved with a razor blade followed by the application of antiseptic iodine solution. Subcutaneous infiltration anesthesia (4 ml per region of mepivacaine 2% with epinephrine 1:100.000) was applied for increased local hemostasis.

In study V, sedation of the minipigs was achieved with an i.m. injection of dexmedetomidine (10 µg/kg; DexDomitor®, Orion Corporation, Espoo, Finland), butorphanol (0.1 mg/kg; Dolorex®, MSD Animal Health, Wellington, New Zealand), midazolam (0.5 mg/kg; Dormipnol®, VioFar LTD, Acharnes, Greece) and ketamine chlorate (3 mg/kg; Ketamidol®, Richter Pharma AG, Wels, Austria). General anesthesia was induced with an intravenous infusion of propofol (0.5 - 1.5 mg/kg; Propofol®, MCT/LCT Fresenius, Fresenius Kabi, Uppsala, Sweden) followed by isoflurane (1 – 2%; Iso-vet®, Piramal Healthcare UK Limited, Morpeth, UK) and maintained in oxygen through an endotracheal tube. The animals were treated for analgesia with a constant rate infusion (CRI) of fentanyl (0.05 µg/kg/hour; Duragesic®, Janssen Cilag AEBE, Thessaloniki, Greece). Intraoperative local anesthesia of xylocaine with adrenalin (20 mg/ml + 12.5 mg/ml, 3.6 ml each side; Xylocain® adrenalin, Astra AB, Södertälje, Sweden) was given to reduce the dosage of systemic analgesic, as well as to increase hemostasis and provide immediate postoperative pain control.

Teeth extraction

In study V, all mandibular premolars (P1 - P4) and the first molar (M1) of the minipigs were extracted bilaterally following segmentation of the teeth by means of a diamond bur on a high-speed hand-piece with internal irrigation. Intraoperative digital periapical radiographs were taken to control the absence of root remnants. The edentulous ridges were allowed to heal for 12 weeks.

Implant insertion

All implants were inserted with a rotation of 30 rotations per minute (rpm). In study I and II, surgical procedures were performed in the same manner in tibia of dogs (Figure 33a). An incision ~5 cm in length was made through the skin and the muscles and periosteum were elevated to expose the bone. Two pairs of implants from each of the experimental groups were placed in each tibia with an alternating fashion in terms of medio-distal positioning regarding the group, but with the first group chosen at random, and with inter-implant distances of 1 cm. The first implant was inserted about 2 cm below the femoral-tibial-patellar joint line at the central medial-lateral portion of the proximal tibiae (Figure 33b). Surgeries were staged between left and right tibia to provide healing times of 2 and 4 weeks.

In study V, a mid-crestal incision in both sides of the edentulous posterior mandible of minipigs (Figure 33c) was made to raise full thickness buccal and lingual flaps. Aiming a more accurate implant placement, the residual alveolar ridge was flattened by means of rotating instruments under copious sterile saline irrigation. Four implants were inserted in each side of the mandible ($n = 8$ implants/animal; Figure 33d). In order to guarantee selection blindness during the experimental allocation, control and experimental groups were randomly distributed in the mandible arch of each minipig in a split-mouth design, according to a computer-generated randomization list. Implants were positioned with their collar on the bone level as per clinical inspection and with a standardized inter-implant distance of 0.5 cm, measured with a metal stent.

Maximum ITV was recorded for each implant at the time of implantation by means of a manual torque wrench. In study I and II, ITV of around 45 N.cm was recorded for all implants, while in

study V, ITV was significantly different between test and control groups ($P = 0.016$), yielding values lower or above the recommended value by the manufacturer (35 N.cm) but not exceeding the maximum value of 60 N.cm (Figure 34).

Figure 33. Illustrations of the animal models and images of the surgical procedures. (a) Lateral view of the canine skeleton. Image courtesy of ©Dave Carlson/CarlsonStockArt.com. (b) In study I and II, two pairs of implants from each of the experimental groups were placed in each tibia with inter-implant distances of 1 cm. Standard cover screws were connected to all implants in study I and II to allow submerged healing for 2 and 4 weeks. This image was adapted from de Jesus et al. (2018)⁶²² and it is licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0). (c) Minipig skeleton, lateral view. Image courtesy of ©Markus Rydén. (d) In study V, four implants were randomly inserted in each side of the mandible following a split-mouth design with a standardized inter-implant distance of 0.5 cm. Standard transmucosal healing abutments were connected to 20 implants in study V to allow non-submerged healing deprived of actual function, while custom-made abutments were connected to other 20 implants to allow for immediate loading.

Standard cover screws were connected to all implants in study I and II to allow submerged healing. Standard transmucosal healing abutments (3.5 x 4.5 mm, L x Ø) were connected to 20 implants in study V to allow non-submerged healing deprived of actual function, while custom-made abutments (8.0 x 4.5 mm, L x Ø) were

connected to other 20 implants to allow for immediate functional loading. The soft tissue was repositioned, appropriately adapted, and sutured with Vicryl 4-0 (absorbable; Ethicon GmbH, Norderstedt, Germany) and Mononylon 4-0 (non-absorbable, Virbac do Brasil Indústria e Comércio Ltda. - studies I and II) for primary intention healing.

Figure 34. Mean $\pm$ s.d. and box plots of the data on ITV (study V). Data represents the interquartile range (IQR; box) between the lower and upper quartile, median (white line), minimum and maximum values (horizontal lines outside the IQR). ITV, insertion torque values.

Drilling protocols

Drilling procedures were performed with a speed between 800 and 1.200 rpm depending on the type of bone, under copious sterile saline irrigation.

In study I and II, bone drilling was similar and followed the manufacturer's instructions (standard drilling, SD). The drilling technique comprised a 6-step series of pilot and multi-fluted twist drills (Figure 35).

In study V, drilling protocols were defined according to the implant diameter and thread dimensions aiming to yield an IDD of

0.3 mm (SD, control group; Figure 36a) and 0.1/0.0 mm (OD, test group; Figure 36b-c), respectively.

SD comprised a 3-step series of 2-mm pilot, 2-mm twist, and 3.2-mm multi-fluted tapered drills without profiling of the cortical bone (IDD = 0.3 mm in the total 11.5-mm length of the osteotomy) to create a press-fit condition with the dimension of the site nearly matching the inner implant diameter while the threads engage within the bone.

OD comprised a 5-step series of 2-mm pilot, 2-mm twist, 3.2-mm and 3.4-mm multi-fluted tapered drills (IDD = 0.1 in the 8-mm central apical length of the osteotomy) in addition to profiling of the cortical bone by employing a 3.5-mm pilot drill (IDD = 0.0 mm in the 3.5-mm coronal length), matching the implant outer diameter.

Figure 35. Graphic illustrations of the surgical preparation of the implant site performed in the tibia of Beagle dogs (study I and II). (a) The drilling technique comprised a 6-step series of 2.0- and 3.0-mm pilot drill drills, 2.0-, 3.0- and 3.3-mm multi-fluted twist drills, and (b) 3.9-mm final pilot drill. The images of the implants were adapted from Neodent®, Curitiba, PR, Brazil.

Figure 36. Graphic illustrations of the surgical preparation of the implant site performed in the minipig mandible (study V). (a) Standard drilling (SD, control group) comprised a 3-step series of 2-mm pilot, 2-mm twist, and 3.2-mm multi-fluted tapered drills without profiling of the cortical bone (IDD = 0.3 mm in the total 11.5-mm length of the osteotomy) to create a press-fit condition. (b) Oversized drilling (OD, test group) comprised a 5-step series of 2-mm pilot, 2-mm twist, 3.2-mm and 3.4-mm multi-fluted tapered drills (IDD = 0.1 in the 8-mm central apical length of the osteotomy) in addition to (c) profiling of the cortical bone by employing a 3.5-mm pilot drill (IDD = 0.0 mm in the 3.5-mm coronal length). IDD, relative implant-last drill discrepancy. These images were adapted from Neodent®, Curitiba, PR, Brazil.

Post-operative care

No pre- or intra-operative complications were observed. I and II, postoperative pain control was achieved with a single dose of ketoprofen (1% i.m.; Ketoprofeno®, Laboratórios Vencofarma do Brasil Ltda., Londrina, PR, Brazil). Post-operative infection control was achieved with local application of Rifampin spray (SEM S.A., São Bernardo do Campo, SP, Brazil) and systemic antibiotics (30.000 IU/kg i.m.; Pentabiotic®, Fort Dodge Saúde Animal Ltda.)

for seven days. In study V, the animals were treated for analgesia with meloxicam (0.4 mg/kg/day; Loxicom®, Norbrook Laboratories, UK) for 3 days.

All animals recovered without any unforeseen incident during the subsequent healing periods of 2 and 4 weeks in study I and II, and 12 weeks in study V. Termination was performed by inducing cardiopulmonary arrest with an intra-cardiac injection of anesthetic overdose under sedation. Bone blocks containing the implants in the upper third of the dog tibias and the minipig mandible were retrieved and fixed by immersion in 10% buffered formalin solution for 2 weeks. In study I and II, following removal of the overlying soft tissues, half of the units were referred to histomorphometrical evaluation (study I) and the other half to biomechanical analysis (study II). In study V, out of 40 implants in total, 10 non-submerged implants were analyzed – the remaining 30 devices are related to a different macrogeometry and loading condition.

***Ex vivo* analytical procedures and methods**

Ex vivo analyses were performed in all, randomly selected, retrieved samples by one blinded calibrated examiner (R.N.R.J.).

Non-decalcified histotechnical procedures

The 2D analysis of parameters of osseointegration at the tissue level requires the application of non-decalcified histotechnical procedures that fully preserve the micro-architecture and structural integrity of the peri-implant mineralized bone. The cutting-grinding technique (EXAKT system), first described by Donath and Breuner (1982)⁵⁶⁵, is the major non-decalcified technique applied for the preparation of section allowing the description and measurement of the rate of bone formation and degree of maturation under light microscopy⁵⁶⁶. This method has another major advantage in preventing shrinkage phenomena of cellular and non-cellular components of the bone marrow, compared to decalcified techniques. Thin ground-sections are created through four major stages: fixation, embedding, cutting-grinding, and staining⁵⁶⁶.

In study I and V, bone blocks containing a single implant were produced, rinsed in running water for 24 h, and gradually dehydrated in a series of ascending concentrations of alcohol from 70%

to 100% ethanol and embedded in methyl-methacrylate based resin (Technovit® 7200, VLC, Heraeus Kulzer®, Hanau, Germany). Subsequently, the samples were photopolymerized and hard blocks were obtained.

Two to three central longitudinal sections from each implant, approximately 300 µm thick, were cut in a direction perpendicular to the diaphysis (study I) and in a mesio-distal direction, parallel to the long axis of the implant (study V), in a thin section cutting technology (EXAKT Advanced Technologies GmbH, Norderstedt, Germany), in duplicate. In the latter study, cutting plane definition was enabled by examination of digital peri-apical radiographs of the embedded hard blocks. Thereafter, the ground sections were grounded and polished to a final thickness of 24 µm on average following previously described protocols using a desktop grinding-polishing unit (EXAKT).

Finally, the sections were stained alternately with toluidine blue (study I and V) or van Gieson's picrofuchsin (study I), and imaged at an incandescent light microscope (Leica DM2500M, Nikon Corporation Instruments Company®, Kawasaki, Kanagawa, Japan) or at a desktop digital slide scanner (NanoZoomer-SQ Digital Pathology System, C13140-01, Hamamatsu®, Hamamatsu, Japan) connected to a computer with dedicated imaging software to create whole images of the biopsy (Imaging Software NIS-Elements, Basic Research, Nikon®, and NDP.serve/NDP.view, Hamamatsu®).

Histomorphometrical analysis of bone-implant interface: parameters assessed

The histomorphometrical measurements were done on the two technically best central mesio-distal sections from each specimen in study I and V.

In study I, in order to exclude any influence on the evaluated parameters from differences in implant site preparation depth and/or implant insertion level, only a central aspect of the implant with the surrounding tissues within the lumen, *i.e.*, excluding the portion of the implant within the cortex, was used in the histomorphometrical evaluation.

First, a rectangular AOI, 6 mm long and extending 0.5 mm on each side of the pitch of the 4th implant thread was superimposed

over the implant image parallel to its long axis and just below the cortical bone (Figure 37). The AOI included a net of 8 vertical (*i.e.*, parallel to the implant axis) and 26 horizontal (*i.e.*, perpendicular to the implant axis) equidistant lines.

Figure 37. Representative histomicrographs illustrating the histomorphometrical analysis of bone-implant interface in study I. (a) A rectangular AOI (in yellow) was superimposed over the image parallel to the long axis. **(b)** BIC was calculated by counting the total number of horizontal lines “hitting” the implant where bone was in direct contact with it. Bone density was evaluated by counting the number of horizontal-vertical line intersections (points) over the mineralized bone. AOI, area of interest. BIC, bone-to-implant contact. Overview at x20 and high magnification at x200. Van Gieson’s picrofuchsin stain. These images were reproduced from de Jesus et al. (2017)⁶²³, ©John Wiley and Sons, Inc (license number: 4543110190163).

The following parameters were measured:

1. Bone-to-implant contact (BIC; %) by counting the number of horizontal lines “hitting” the implant where the bone was in direct contact with it, expressed as a percentage of the total number of horizontal line-implant intersections.

2. Peri-implant bone density (BD; %) by counting the number of horizontal-vertical line intersections (points) over mineralized bone, expressed as a percentage of the total number of points within the AOI but excluding the points over the implant.

In study V, two rectangular AOI were determined to represent the central coronal (7 mm, L) and apical aspects of the implant (4.5 mm, L) due to its dual-tapered body and dynamic thread design (AOI-c and AOI-a, respectively; Figure 38a). The following parameters were measured:

1. The distance from the implant platform to the first coronal mineralized BIC (f-BIC; mm) at the mesial (f-BIC/M) and distal sites (f-BIC/D), representing the marginal peri-implant bone level (MBL): the height of marginal bone resorption provided that implants were positioned with their collar on the bone level and that occurrence of bone defects (*e.g.*, buccal dehiscence) were adequately recorded.
2. BIC at both AOI (BIC-c and BIC-a) and at the total implant length (BIC-len; Figure 38b-d).
3. BD: the amount of bone in a specific reference area extending 0.5 mm laterally from the implant encompassing the peri-implant tissues in both AOI-c and AOI-a (BD-c and BD-a, respectively) and on the total implant length (BD-len). Because of the dissimilarities in IDD mainly in the central coronal region, BD-c was analyzed within the threads [BD-c/Th] and on the implant perimeter [BD-c/Pe]; the total amount ($BD-c/T = BD-c/Th + BD-c/Pe$) was then calculated (Figure 38d).

Assessment of interfacial biomechanical properties of osseointegrated implants: parameters assessed

The biomechanical strength of the bone-implant interface is usually analyzed by assessing the maximum removal torque value (RTV), the maximum force required to loosen the interfacial strength⁵⁶⁷. Although this parameter is important, use of a single parameter appears not sufficient for a complete biomechanical assessment of osseointegration. Herein, connection stiffness, reflecting the rigidity

(*i.e.*, the stability of the implant under load)²⁹⁶, and removal energy, reflecting the overall energy (workload) necessary to loosen the bone-to-implant connection⁵⁶⁸, were additionally assessed for complete evaluation of the biomechanical properties.

Figure 38. Representative micrographs illustrating the histomorphometrical analysis of bone-implant interface in study V. (a) Two rectangular AOI represent the central coronal and apical aspects of the implant (AOI-c and AOI-a, respectively; red dashed lines). The distance from the implant platform to the f-BIC (mm; blue lines) represents the MBL. Overview at x20. Scale bar: 1 mm. (b-d) Images at a higher magnification illustrate BIC (%) (yellow lines) and BD (%) 0.5 mm laterally from the implant encompassing the peri-implant tissues in both (b, c) AOI-c and (d) AOI-a and on the total implant length (BD-len). Scale bar: 250 μ m. Color code: (b, d) purple, total amount of BD; (c) red, BD within the Th; blue, BD on the Pe. a, apical region of the implant. AOI, area of interest. BD, bone density. BIC, bone-to-implant contact. c, central coronal region of the implant. f-BIC, first bone-to-implant contact. len, total implant length. MBL, peri-implant marginal bone level. Pe, implant perimeter. Th, implant threads. Toluidine blue stain.

Thus, in study II, the following biomechanical parameters were assessed:

1. Maximum removal torque (N.cm): obtained during the unscrewing process (primary outcome measure).
2. Connection stiffness (N.cm/rad): the ratio between removal torque and angular displacement (secondary outcome measure).
3. Removal energy ($\times 10^{-2}$ J): the energy (workload) necessary to completely unscrew the implant (secondary outcome measure).

The removal torque test (RTQ) was conducted on a Shimadzu universal testing machine. This equipment was adapted in order to determine the referred properties. A horizontal shaft, supported by two ball bearings, with Allen keys socket on one end and a rotation sensor on the other end, was connected with a steel string to the mobile span of the Shimadzu universal testing machine, in such a way that the linear motion was converted to a rotational motion. The dog's tibia bone block containing the implant was placed in alignment and inside the Allen keys socket and fixed with an adjustable clamp, in order not to rotate during the test (Figure 39a). The upper span speed, at which the string was attached, was adjusted to produce a shaft rotation speed of 0.005 rad/s. During the test, both torque and angular displacement were acquired using a sampling rate of 10 samples/s (Figure 39b-c).

In order to calculate the connection stiffness, the tangent method was applied to the data after obtaining the linear correlation coefficient (R^2) compared to the secant method, revealing the absence of mathematical discrepancy between both methods. Two different osseointegrated implants can have the same maximum RTV, but distinct connection stiffness; similarly, they may show the same removal energy but perform in a very distinct fashion and show distinct connection stiffness and maximum RTV.

Statistical analysis

By using mathematical models, it is possible to test the statistical significance of a parameter inferred from a predicted (dependent) variable and a specific predictor (independent) variable⁵⁶⁹. Numer-

ous tests were applied in the studies included in this thesis for interpretation of the quantitative, continuous data (numeric values set on a scale) generated by a causative process of interest.

Figure 39. A graphic illustration and representative curve of the RTQ of os-seointegrated implants in study II. (a) Assembly detail of the connection between Allen keys socket in the Shimadzu universal testing machine and the implant placed in the tibia of a Beagle dog. (b) Graph of torque versus angular displacement with linear regression curve and equation representing the connection stiffness. (c) Determination procedure of unscrewing implant work up to test's maximum torque. RTQ, removal torque test. These images were reproduced from de Jesus et al. (2018)⁶²² and they are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0).

First, the frequency distribution of the data was assessed (parametric or non-parametric) based on the manner it is scattered along the scale by submitting the data to normality test (Shapiro-Wilk) and equal variance test (Levene). In all studies, quantitative data were expressed as mean $\pm$ standard deviation (s.d.; parametric data) and median (non-parametric data). Then, general linear statistical models were applied for significant differences between variables quantified for each parameter.

In study I, implants from control (SAE) and test groups (SAE-HD) at two observation periods (2 and 4 weeks) were placed in the same animal. Considering its non-parametric distribution, differences between the median of the two dependent samples were tested for significance with the Wilcoxon signed-rank test. Rank-order statistical methods are used to test the difference (stated as positive or negative) between two dependent samples, which is ranked in a defined order, from the smallest to the highest. Finally, the test calculates and compares the mean of the ranks between SAE-HD and SAE.

In study II, one-way analysis of variance (ANOVA) was performed to determine whether there were any statistically significant differences between the means of three or more samples (control vs. test, measured over time) assuming a parametric distribution for all the evaluated parameters. In case of detecting a difference, the Tukey *post-hoc* test was performed for multiple comparisons to identify which group revealed a significant difference.

In study III, at least three independent experiments were performed for each assay. MTT optical density and TRAP activity values were compared by ANOVA, followed by Turkey's *post hoc* test for multiple comparisons with the value of statistical significance set at $P < 0.001$. For the gene expression analysis, the aforementioned test was performed for comparison of TRAP1 mRNA levels, followed by Dunn's *post hoc* test for comparisons with the positive control group ($P < 0.05$). Due to the asymmetrical distribution of data for gene expression levels of CTSK, MMP9, CALCR, ARG1, IL6, TNF and BAX, cell size and actin ring size, a Kruskal-Wallis non-parametric ANOVA was used, followed by Dunn's *post hoc* test for comparisons with the control group, CCPC (+) ($P < 0.05$ and $P < 0.001$, respectively).

In study V, the Mann-Whitney test and the independent t-test were applied for significant differences between two dependent samples (SD vs. OD) presenting nonparametric and parametric data, respectively.

The Spearman's Rank-Order (Rho) test was taken to evaluate the monotonic relationship between two continuous variables – between RTV and its intrinsic properties (removal energy and connection stiffness) in study II, and between ITV and the parameters of osseointegration in study V. A monotonic relationship occurs when two variables may be altered simultaneously to the same direction, either to higher or lower values, and is based on the ranked values rather than on the raw data. A correlation coefficient (r) may vary between low and strong, negative or positive, where a value of 1 means a perfect positive correlation and the value of -1 means a perfect negative correlation.

In study I, II, and V, the level of significance was set at $P < 0.05$. Parametric tests were all two-tailed (two-sided), which implies that the estimated mean value may vary between lower or higher values compared to a reference average value. Repeated measurements for data reproducibility (*i.e.*, accuracy and precision) of BIC and connection stiffness were performed from 8 randomly selected implants, with a 1-week interval. Due to small sample size, a *post-hoc* analysis was performed to define the minimum detectable difference between groups regarding the parameters assessed (BIC and RTV), with a power of 80% and an alpha error of 0.05%.

For all the analysis in study I, II, III, and V, the IBM SPSS Statistics software was used (IBM Company Inc., Chicago, IL, USA).

Meta-analysis

Meta-analysis is the statistical analysis of data from several controlled studies comparing different interventions, pooled to one mean value, which present a low to moderate risk of bias and well-controlled variables⁵⁷⁰. This method is considered a reliable approach to reduce random error and increase precision due to resulting larger sample size and smaller 95% CI⁵⁷¹.

In study IV, a meta-analysis was performed to compare biomechanical and biological outcomes of oversized (experimental) and standard/undersized (control) surgical preparation of the implant

site by means of biomechanical (ITV and RTV) and histomorphometric (%BIC and %BD) data [means $\pm$ s.d. and number of animals (n) per group] in small and large animal models. The DerSimonian and Laird random effects model was applied using STATA Statistical Software (StataCorp LP, College Station, TX, USA) to determine if one approach has statistical superiority compared to the other⁵⁷¹.

Since the studies included multiple interventions per animal (*i.e.*, split-mouth design), a correlation coefficient (R-value of 0.50) was applied for between-treatment group measurements⁵⁵⁵. Pooled estimates of treatment effect (weighted mean difference [WMD]; *i.e.*, mean of the test group minus mean of the control group divided by the pooled s.d. of both groups) and 95% CI were calculated.

Statistical heterogeneity was assessed by graphic display and by determining the consistency of the WMD from the included studies in conjunction with 95% CI. The chi-square test was used to assess heterogeneity, which classification was established as low (25%), moderate (25%), and high (25%) degree. The test for homogeneity (I^2) was assumed to quantify the extent of heterogeneity as a measure of inconsistency of results across studies. Forest plots were generated for analysis of data and significance level was set at $P < 0.05$.

SUMMARY OF RESULTS

If the result confirms the hypothesis, then you've made a measurement.

If the result is contrary to the hypothesis, then you've made a discovery

Enrico Fermi (1901 - 1954), Italian physicist

The effect of hydrophilicity on early osseointegration and biomechanical properties of titanium implants *in vivo* (Study I & II)

In study I and II, *in vivo* analyses were performed to evaluate the respective histomorphometrical parameters (BIC and BD) and biomechanical properties (RTV, removal energy, and connection stiffness) at implants with a sandblasted, dual acid-etching, with or without an additional chemical modification increasing hydrophilicity (SAE-HD [test] and SAE [control], respectively) at early stages of osseointegration (2 and 4 weeks) in the tibia of Beagle dogs.

Descriptive histological results

In general, new bone formation along, and in contact with, the implant surface could be observed irrespective of the experimental group and observation period, however, with some variation among implants (Figure 40). Some variation was observed regarding the depth of implant site preparation and implant insertion level, as well as in regard with the shape of the tibia (triangular or oval) at the site of implantation. With regard to the latter, no sig-

nificant difference (NSD) was observed regarding the distribution of implants between the SAE-HD and SAE groups.

At the 2-week observation period, slender WB trabeculae had formed in contact with a portion of the implant surface and in continuation with the inner cortex of the tibia (Figures 40a-b). After 4 weeks of healing, a large portion of the implant surface was in contact with bone and trabecular bone filled a large portion of the lumen itself (Figures 40c-d). Most of the bone was woven but small quantities of LB, mainly in close proximity to the cortex could also be observed.

Figure 40. Representative histomicrographs of experimental and control groups at both healing times. (a, c) Hydrophilic SAE-HD and (b, d) hydrophobic SAE implants at 2 weeks (upper row) and 4 weeks (lower row) *in vivo* (study I). No remarkable differences can be observed between groups and between observation periods regarding BIC and BD. Most of the bone was woven but small quantities of lamellar bone could also be observed, mainly at the 4-week observation time. BD, bone density. BIC, bone-to-implant contact. Overview at x20 and high magnification at x200. Van Gieson's picrofuchsin stain. These images were reproduced from de Jesus et al. (2017)⁶²³, ©John Wiley and Sons, Inc (license number: 4543110190163).

In cases where a large part of the implant was in close proximity to the inner aspect of the cortex; reduced bone formation was ob-

served at 2 weeks. After 4 weeks, however, the extent of bone formation appeared similar to that in the rest of the specimens.

Histomorphometric results

The results of the histomorphometric evaluation are presented in Table 5 and Figures 41a-b. Similar amounts of BIC and BD were observed in both groups (SAE-HD vs. SAE) at each observation time, and there were no statistically significant differences regarding BIC and BD between the groups at any observation period ($P > 0.05$). Based on the observed results, only a rather large difference in BIC (37%) between the two groups would have been possible to disclose with enough power (80%) and the current sample size.

TABLE 5 Mean $\pm$ s.d., median, and 95% CI of mineralized bone-to-implant contact (BIC, %) and bone density (BD, %) for SAE-HD and SAE implants at 2- and 4-weeks post-operatively. Differences were tested for statistical significance with the Wilcoxon test ($P < 0.05$)

Time in vivo	SAE-HD			SAE			P value
	Mean $\pm$ s.d.	Median	95% CI	Mean $\pm$ s.d.	Median	95% CI	
BIC (%)							
2 w	19.57 $\pm$ 13.57	16.63	5.32 - 33.81	20.33 $\pm$ 7.99	19.05	11.94 - 28.71	0.75
4 w	42.80 $\pm$ 14.48	40.95	27.62 - 58.31	40.25 $\pm$ 9.45	36.48	30.33 - 50.17	0.65
BD (%)							
2 w	24.85 $\pm$ 16.31	21.75	7.73 - 41.97	25.66 $\pm$ 8.58	24.65	16.65 - 34.67	0.34
4 w	44.13 $\pm$ 6.46	42.40	37.35 - 50.91	40.13 $\pm$ 6.46	40.38	35.38 - 44.87	0.25

Figure 41. Histomorphometrical data of hydrophilic SAE-HD versus hydrophobic SAE implants at 2 and 4 weeks *in vivo*. Mean $\pm$ s.d. and dot plots of the data on percentage **(a)** BIC and **(b)** BD ($P > 0.05$). BD, bone density. BIC, bone-to-implant contact. These images were reproduced from de Jesus et al. (2017)⁶²³, ©John Wiley and Sons, Inc (license number: 4543110190163).

Biomechanical results

The biomechanical properties were assessed by means of maximum RTV, removal energy, and connection stiffness values at SAE-HD and SAE implants at 2- and 4-weeks post-operatively.

SAE-HD implants resulted in greater RTV (N.cm; Figure 42a) and removal energy ($\times 10^{-2}$ J; Figure 42b) compared to SAE at 2 and 4 weeks; however, there was no statistically significant differences between groups at any observation period ($P = 0.06$ and $= 0.16$, respectively) but a slight increase over time in both test and control groups.

With regard to connection stiffness (N.cm/rad; Figure 42c), SAE revealed the lowest value at 2 weeks, however, not statistically significant neither over time nor between groups ($P = 0.76$). SAE-HD disclosed nearly similar results at both healing periods.

The Spearman's correlation coefficient (r) was obtained to determine the relationship between parameters. There was a strong, positive monotonic correlation between removal torque and removal energy ($r = 0.722$, $P < 0.001$). However, there was no evidence of positive correlation between removal torque and connection stiffness ($r = 0.352$, $P = 0.092$).

Figure 42. Biomechanical properties of hydrophilic SAE-HD versus hydrophobic SAE implants at 2- and 4-week observation periods. Mean $\pm$ s.d. and dot plots of the data on (a) RTV, (b) removal energy, and (c) connection stiffness ($P > 0.05$). RTV, removal torque values. These images were reproduced from de Jesus et al. (2018)⁶²² and they are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0).

The effect of hydrophilicity and implant macrodesign on osteoclast differentiation, activation, and survival in a murine macrophage-like cell model (Study III)

In study III, *in vitro* analyses were performed to evaluate the influence of the implant thread design (trapezoidal vs. triangular threads) and surface hydrophilicity (SAE-HD vs. SAE) on osteoclast (OC) differentiation, activation, and survival in vitro in a model of RAW 264.7 cells. Titanium disks presenting different surfaces and macrodesign were produced. Macrophages were differentiated to OC and assessed by enzymatic TRAP activity and MTT assay. Expression levels of various OC-related genes were measured by qPCR, in addition to qualitative and quantitative image analysis of OC proliferation, adhesion, and morphology by means of SEM and CLSM.

Osteoclast differentiation and cell viability

Following 5 days of RANKL-induced osteoclast differentiation (RAW-OC formation), hydrophilic surfaces (HLX/SAE-HD and TMX/SAE-HD) significantly suppressed osteoclast differentiation ($P < 0.01$) when compared to the positive control group [CCPC (+)] (Figure 43a). Similarly, HLX/SAE resulted in significantly reduced TRAP activity; while TMX/SAE group did not have any influence on the modulation of OC differentiation when compared to CCPC (+) ($P > 0.05$). CCPC (-) exhibited the minimum enzymatic activity compared to CCPC (+), TMX/SAE, and HLX/SAE-HD ($P < 0.001$), HLX/SAE ($P < 0.01$) and TMX/SAE-HD ($P < 0.05$).

The results of the MTT assay showed significantly different absorbance levels among the experimental groups ($P < 0.001$; Figure 43b); specifically, increased viability in TMX/SAE disks and reduced viability in HLX/SAE-HD disks were observed. LPS induced OC activation and proliferation ($P = 0.001$) when compared to CCPC (+) and, therefore, used as control for gene expression of cytokine levels, being a stimulus that induces cytokine production.

Figure 43. Mean ± s.d. and dot plots of the data on differentiation and viability of RAW-OC cells following 5 days of culture. (a) OC differentiation assessed with an enzymatic TRAP activity assay. (b) Cell viability. Dissimilar letters indicate statistically significant differences at 0.05 (*), 0.01 () and 0.001 (***) among groups. OC, osteoclast. TRAP, Tartrate-Resistant Acid Phosphatase.**

Osteoclast-specific gene expression

The following osteoclastogenesis and cell survival related genes did not show any statistically significant differences compared to CCPC (+): TRAP1, CTSK, MMP-9, CALCR, ARG1 and BAX ($P > 0.05$; Figures 44a-e, h). The only significant difference was in expression levels of genes associated with osteoclast-related inflammatory cytokines, when compared to the positive control group. Specifically, expression of IL-6 and TNF was more than 6-fold and 19-fold upregulated in TMX/SAE-HD, respectively ($P < 0.05$ and $P < 0.01$; Figures 44f-g).

Osteoclast proliferation, adhesion, and morphology

RAW-OC differentiation, adhesion, and morphological structures were investigated on different surface technologies presenting HLX and TMX macrogeometry (Figures 45 and 46, respectively) by means of CLSM and SEM. Magnified representative confocal micrographs, with cells immunoassayed for F-actin (cytoskeleton) and DNA (cell nuclei), demonstrate distinctive adhesion structures formed by RAW-OC cells on experimental Ti disks. Cells cultured on all substrates formed extensive podosomes – actin-derived structures associated with integrin-mediated cell adhesion. Single podosomes as well as actin rings were mainly distributed along the cytoplasm of cells differentiated on SAE-HD surfaces (Figures 45a and 46a), whereas predominantly larger but not necessarily more actin rings were formed on SAE (Figures 45g and 46g). SEM images allowed the assessment of cellular adhesion and proliferation (Figures 45 and 46, b-f and j-l).

Figure 44. Mean $\pm$ s.d. and dot plots of the mRNA gene expression levels. (a-c) Osteoclastogenesis, osteoclast activity and (d-e) its negative modulation, (f-g) pro-inflammatory status, and (h) cell survival following 5 days of osteoclast differentiation on the negative control [CCPC (-)], positive control [CCPC (+)] groups, experimental Ti disks (hydrophilic SAE-HD and hydrophobic SAE surfaces presenting either a dynamic progressive thread geometry [HLX] or double triangular threads [TMX]), and assay control group (CCPC-LPS). Results are presented as relative normalized expression of three independent experiments including samples in triplicate and two technical replicates. Asterisks indicate statistically significant differences at 0.05 (*), 0.01 (**), 0.001 (***) compared to CCPC (+).

Figure 45. Qualitative image analysis of osteoclast proliferation, adhesion, and morphology of RAW-OC cells following 5 days of differentiation on HLX disks with a dynamic progressive thread geometry, by means of CLSM and SEM. (a, g) Magnified representative confocal micrographs demonstrate distinctive adhesion structures. **(b-f)** Cells were randomly distributed in small clusters on top of the thread tips and flanks of hydrophilic SAE-HD surfaces, while compact cell assembly was identified predominantly in the valley. **(h-l)** Relative to hydrophobic SAE surfaces, clusters of OCs were identified along the entire pitch area of the thread design. **(e-f, k-l)** SEM analysis at high magnification revealed similar tightened adhesion structures and filopodia projections of osteoclasts differentiated on both surface technologies. Color code: (a, g) green, actin cytoskeleton; blue, cell nuclei. CLSM, confocal laser scanning microscopy. OCs, osteoclasts. RAW-OC cells, macrophages treated for differentiation to osteoclasts. SEM, scanning electron microscopy.

TMX/SAE-HD

TMX/SAE

Figure 46. Qualitative image analysis of osteoclast proliferation, adhesion, and morphology of RAW-OC cells following 5 days of differentiation on TMX disks with double triangular threads, by means of CLSM and SEM. (a, g) Magnified representative confocal micrographs demonstrate distinctive adhesion structures. **(b-f)** Cells were randomly distributed in small clusters on top of the thread tips and flanks of hydrophilic SAE-HD surfaces, while compact cell assembly was identified predominantly in the valley. **(h-l)** Relative to hydrophobic SAE surfaces, clusters of OCs were identified along the entire pitch area, mainly in the valleys of the thread design. **(e-f, k-l)** SEM analysis at high magnification revealed similar tightened adhesion structures and filopodia projections of osteoclasts differentiated on both surface technologies, without further influence of the macrogeometry when compared to HLX. Color code: (a, g) green: actin cytoskeleton; blue: cell nuclei. CLSM, confocal laser scanning microscopy. OCs, osteoclasts. RAW-OC cells, macrophages treated for differentiation to osteoclasts. SEM, scanning electron microscopy.

In regard to quantitative analyses of OC adhesion and morphology, RAW-OC formation on HLX/SAE and TMX/SAE revealed the greatest range in size compared to HLX/SAE-HD, TMX/SAE-HD, and CCPC (+) ($P < 0.001$; Figure 47a). Actin rings were larger in TMX/SAE, however NSD was detected between groups ($P \geq 0.05$; Figure 47b).

Figure 47. Box-plots of the quantitative image analysis of RAW-OC cells proliferation and adhesion following 5 days of differentiation on the experimental titanium disks. (a) Cell size. (b) Actin ring size. Data represents the interquartile range (IQR; box) between the lower and upper quartile, median (white line), minimum and maximum values (horizontal lines outside the IQR). Dissimilar letters indicate statistically significant differences at 0.001 (***). RAW-OC cells, macrophages treated for differentiation to osteoclasts.

The effect of oversized surgical preparation of the implant site on osseointegration of titanium implants in experimental animal models (Study IV & V)

The effect of oversized surgical preparation of the implant site on parameters of osseointegration of titanium implants was assessed by means of a systematic review and metanalysis of preclinical animal studies (study IV), and an *in vivo* evaluation (study V).

In study IV, the current evidence on the effects of oversized surgical preparation of the implant site in terms of biomechanical (ISQ, ITV, and RTV) and biological parameters of osseointegration (BIC and BD) in experimental animal models was systematically assessed and a meta-analysis of the estimate of efficacy of oversized (test OD, IDD = 0.0 – 0.1 mm; and test GAP, IDD ≤ -0.1 mm) vs. standard or undersized surgical preparation of the implant site (control, IDD ≥ 0.2 mm) was performed.

In study V, histomorphometrical evaluation of the effect of oversized surgical preparation of the implant site (OD, IDD = 0.1 mm in the 8-mm central apical length of the osteotomy and IDD = 0.0 mm in the 3.5-mm coronal length) on MBL and histomorphometrical parameters of osseointegration (BIC and BD) at tapered implants with a progressive thread geometry was performed compared to a standard surgical preparation (SD, IDD = 0.3 mm) following 12 weeks *in vivo* in the minipig mandible.

Systematic review: study selection and characteristics

The PRISMA flow chart of the present systematic review are illustrated in Figure 48. The initial electronic search strategy resulted in hypothetically applicable 990 records. Following exclusion of duplicates and the first-stage screening of titles and abstracts, 20 full-text articles, which were assessed for eligibility according to the inclusion and exclusion criteria. Following the full-text assessment, 12 articles were included in the present systematic review.

Overall, studies were of low to unclear risk of bias (Figures and). The majority of the studies adopted dogs as the animal model (58%), followed by sheep (17%), rabbits (17%), and minipigs (8%). Regarding biomechanical analysis, 7 studies investigated the effects of oversized drilling technique on ITV (38%), RTV (24%), and %ISQ (38%) in both intra-oral (24%) and extra-oral sites

(76%). In 10 studies, histomorphometrical parameters were investigated, including %BIC (100%) and %BD (82%) in intra-oral (40%) and extra-oral sites (60%). In total, 11 studies (92%) included SD as the control group, whereas only 5 studies (42%) evaluated UN in comparison to test OD and/or test GAP.

Figure 48. PRISMA flow diagram. Double-blind three-stage screening process in study IV: search strategy, study screening and final selection. Interrater reliability (κ) describes the level of agreement between reviewers. n, number of articles. κ , kappa coefficient.

Descriptive histological results

In study IV, data on descriptive histology of each study was collected and summarized. In general, oversized surgical preparation of the implant site was characterized by healing chambers filled with osteogenic tissue and extensive WB formation in the early healing period, indicating an intramembranous-type healing mode, in comparison to sites in absence of void spaces^{384,398,399,401,483,500–502}. None of the studies found micro-fractures in the peri-implant bone or ischemic necrosis following implant placement in oversized sites. At the late healing period, implants were well osseointegrated into comparable mineralized bone in healing chambers and in contact to the implant surface, regardless of the surgical protocol^{399,466,501,523}.

In turn, standard and undersized drilling techniques resulted in collagen deformation and microcracks in the cortex³⁹⁸, extensive areas of necrotic bone and substantial remodeling, especially in the primary thread engagement area^{401,500,501,516}.

In study V, histomicrographs of OD and SD groups were assessed for histological analysis. Overall, histological analysis revealed certain variation in terms of osseointegration and MBL among the groups (Figures 49 and 50). All animals presented a relatively dense bone macrostructure with thick trabecular structures and slender medullary space, in spite of a minor interindividual variability. Standard drilling of the implant site resulted in distinct resorption of the marginal bone with subsequent crater formation and partial fibrous tissue encapsulation (FTE). One implant undergone total FTE, while another implant was lost – the latter was inserted with the maximum ITV (60 N.cm; Figures 49a and 50a).

Implants installed with oversized drilling technique revealed adequate osseointegration and reduced marginal bone changes (Figures 49b and 50b). Only one implant yielded partial osseointegration with extensive bone resorption at the mesial site. Remodeling of the surrounding bone in both central coronal and apical regions of the implants was observed in both groups, characterized by the presence of basic multicellular units (BMUs) and cement lines (Figure 49c-f). However, oversized-drilled sites presented with primary osteonic structures within the threads and overall decreased level of remodeling. Peri-implant regions with low to deficient mineralized

tissue exhibited a low to moderate inflammatory infiltrate and bone under active resorption in close proximity to the surface (Figure 51), irrespective of the drilling technique.

Figure 49. Representative histomicrographs comprising standard drilling (SD; control) and oversized drilling (OD; test) of the implant site after 12 weeks of healing in study V. (a) CBL with subsequent crater formation. **(b)** Adequate osseointegration and reduced MBL changes. **(c-d)** Remodeling of the surrounding bone characterized by the presence of BMUs and cement lines. **(e-f)** Low to moderate inflammatory infiltrate and bone under active resorption. Red dashed rectangle, magnified area. Green asterisks, BMUs. Black arrowheads, osteoclasts. Black arrows, bone resorption. Red arrowheads, osteoblasts. Red asterisks, bone formation. White arrowheads, cement lines. White asterisks, osteonic structures. Yellow dotted circumference and asterisks, inflammatory infiltrate. BMUs, bone multicellular units. CBL, crestal bone loss. MBL, peri-implant marginal bone level. Overview at x20. Toluidine blue stain.

(a) Standard drilling (SD)

(b) Oversized drilling (OD)

Figure 50. Representative histomicrographs from all implants at 12 weeks of healing, comprising (a) standard drilling (SD) and (b) oversized drilling (OD) of the implant site in study V. One implant installed with the SD protocol undergone total FTE possibly due to the close proximity to the remaining canine root (< 1 mm), while another implant was lost (implant filled in black); the latter was inserted with the highest ITV (60 N.cm). FTE, fibrous tissue encapsulation. ITV, insertion torque value. Overview at x20. Toluidine blue stain. Scale bar: 1 mm.

Figure 51. A representative micrograph of a peri-implant region with deficient mineralized tissue. Low to moderate inflammatory infiltrate and bone under active resorption (black arrows) in close proximity to the implant surface characterized by the presence of multinucleated cells (orange arrowheads), irrespective of the drilling technique. Toluidine blue stain. Scale bar: 50 mm.

Metanalysis of preclinical *in vivo* studies

Detailed information specific to parameters included in the meta-analysis is shown in the study IV in the end of this thesis. Overall, the meta-analysis revealed statistically significant effects in favor of:

1. Control vs. test OD in short-term healing and extra-oral sites, with pooled estimates (WMD) of 25.35 N.cm ITV (95% CI: 20.94, 29.75, $P = 0.000$; Figure 52a) and 26.19% BD (95% CI: 20.10, 32.27, $P = 0.000$; Figure 53a).
2. Control vs. test OD in long-term healing and intra-oral sites, with WMD of 11.69% BD (95% CI: 6.98, 16.40, $P = 0.000$; Figure 53c).
3. Control vs. test GAP in intermediate-term healing and extra-oral sites, with WMD of 3.03% BD (95% CI: 0.44, 5.63, $P = 0.02$; Figure 53d).
4. Control vs. test GAP in long-term healing and extra-oral sites, with WMD of 5.57 N.cm RTV (95% CI: 1.26, 9.88, $P = 0.000$; Figure 52b).

Figure 52. Forest plot for the comparison of implants installed in sites prepared with standard drilling technique (control; $\text{IDD} \geq 0.2$ mm), stress-free oversized drilling technique (test OD; $\text{IDD} = 0.0 - 0.1$ mm), and friction-free oversized implant drilling technique (test GAP; $\text{IDD} \leq -0.1$ mm). Biomechanical outcomes of (a) ITV (N/cm) and (b) RTV (N/cm), and (c) the biologic outcome of BIC (%). The forest plot displays the relative weight of the individual studies, the estimates of treatment effect (ES) expressed as weighted mean differences (WMD), 95% confidence intervals (CI) and a predictive interval for the outcomes. The diamond indicates the pooled estimate and its 95% CI. BIC, bone-to-implant contact. IDD, relative implant-last drill discrepancy. ITV, insertion torque values. RTV, removal torque values.

%BD - test OD

(a) Short-term healing - extra-oral

(b) Intermediate-term healing - intra-oral

(c) Long-term healing - intra-oral

%BD - test GAP

(d) Intermediate-term healing - extra-oral

Figure 53. Forest plot for the comparison of implants installed in sites prepared with standard to undersized drilling technique (control; $\text{IDD} \geq 0.2$ mm) and stress-free oversized drilling technique (test OD; $\text{IDD} = 0.0 - 0.1$ mm) or friction-free oversized implant drilling technique (test GAP; $\text{IDD} \leq -0.1$ mm) for the biologic outcome of BD (%). (a) Short-, (b, d) intermediate-, (c) and long-term healing. The forest plot displays the relative weight of the individual studies, the estimates of treatment effect (ES) expressed as weighted mean differences (WMD), 95% confidence intervals (CI) and a predictive interval for the outcomes. The diamond indicates the pooled estimate and its 95% CI. BD, peri-implant bone density. IDD, relative implant-last drill discrepancy.

Overall, low ($I^2 = 0\%$) to moderate and high ($I^2 = 53\text{--}88\%$, respectively) heterogeneity was observed in the analyses.

Histomorphometric results

In study V, histomorphometry was performed in the central coronal (AOI-c) and apical aspects of the implant (AOI-a), and its total amount (-len) was calculated for the parameters of BIC, and BD; the latter was measured within the threads (Th), on the implant perimeter (Pe), and its total amount (T) within each AOI.

The results of the histomorphometric evaluation are presented in Figure 54. Statistical assessment of f-BIC (mm) did not reveal any significant differences between OD and SD, neither at the mesial site nor at the distal site (Figure 54a). However, an increase in peri-implant crestal bone loss (CBL) could be observed in standard-drilled implant sites with a rather greater intra-group variation compared to oversized-drilled sites. OD showed statistically significant higher values of BIC at both AOI (BIC-c [$P = 0.04$] and BIC-a [$P = 0.012$]), and at the total implant length (BIC-len [$P = 0.005$]) compared to SD (Figure 54b).

Bone density values surrounding the central coronal aspect of the impact (AOI-c) are reported in Figure 54c. OD showed statistically significant higher bone density values within the AOI-c (BD-c/Th [$P = 0.01$], BD-c/Pe [$P = 0.03$] and BD-c/T [$P = 0.02$]) compared to SD. %BD values surrounding the apical aspect of the implant (BD-a) and the total implant length (BD-len) are reported in Table 6. Similar amounts of BD-a were observed between the groups, without significant differences. OD showed statistically significant higher BD-len (BD-len/Th [$P = 0.003$], BD-c/Pe [$P = 0.03$] and BD-c/T [$P = 0.01$]) compared to SD.

TABLE 6 Total amount of bone density (BD-T, %) within the AOI-a (BD-a) and surrounding the total implant length (BD-len) for both groups

	Standard drilling (SD)		Oversized drilling (OD)		<i>P</i> value
	Mean ± s.d.	Median	Mean ± s.d.	Median	
BD-a/T	44.47 ± 31.49	52.05	71.94 ± 9.66	72.45	0.11
BD-len/T	25.32 ± 17.11	31.86	54.46 ± 10.72	50.51	0.01

Figure 54. Histomorphometrical data of standard drilling (SD; control) and oversized drilling (OD; test) of the implant site after 12 weeks *in vivo* in study V. Mean ± s.d. of the data on **(a)** f-BIC (mm), **(b)** BIC (%) within both AOI, central coronal and apical, and at the total implant length, and **(c)** BD (%) within the AOI-c within the threads, on the implant perimeter, and its total amount. f-BIC did not reveal any significant differences between groups. For all parameters of BIC and BD, oversized drilling resulted in statistically significant higher values compared to standard drilling. AOI, area of interest. BD, peri-implant bone density. BIC, bone-to-implant contact. c, central coronal region of the implant. f-BIC, first bone-to-implant contact.

DISCUSSION

*I was taught that the way of progress
was neither swift nor easy*

Marie Curie (1867 - 1934), French-Polish physicist

Rational for performing preclinical *in vitro* and *in vivo* testing of biomaterials

***In vitro* models to study biomaterial-based modulation of cellular and molecular mechanisms**

Several animal and human *in vitro* cell culture models have been developed to evaluate the biochemistry and physiology of living cells and the molecular and cellular basis of human disease. In the field of material science, strategies primarily intended to examine the material biocompatibility at the initial stages of its development by means of tests of cytotoxicity and genotoxicity prior to any further preclinical studies in experimental animals and clinical trials⁵⁷². Throughout the years, the establishment of a large source of different cell types and culture models enabled the investigation of underlying molecular and cellular mechanisms of osteoimmunology and bone formation, which has extensively been applied in biomedical engineering research⁵⁷³.

A decision on the type of cell, among primary cells or immortalized cell lines, has to be made based on the stage of material development and screening⁵⁷⁴. Cell lines are well-established tumorous

or genetically manipulated cells, isolated from multicellular organisms. They continuously undergo division, providing immediate and abundant availability of homogeneous cells with a stable phenotype. Although cell lines are easy to work with, not requiring further characterization prior to testing, senescence and cloning of non-certified subcultures may result in phenotypic instability, which might no longer represent distinctive features of a specific tissue. Thus, freshly isolated cells, in turn, are preferred over the use of cell lines in certain types of experimental setups. However, methods of selective isolation and characterization of primary cells are technically sensitive, time-consuming, and more expensive.

Overall, *in vitro* studies allow the evaluation of receptor-mediated proteins, signaling pathways, gene expression levels, activation of functional processes and their respective modulatory systems associated with cell attachment, morphology, proliferation, differentiation, and activation. In oral implant research, much focus has been given to the potential of surface properties to modulate the process of osteogenesis *in vitro* by applying osteoblastic cell lines (*e.g.*, human fetal osteoblastic [hFOB 1.19], sarcoma osteogenic [MG-63, HOS TE85, and SaOS-2], and mouse calvaria osteoblastic cells [MC3T3-E1])⁵⁷⁵.

In turn, isolation of human primary osteoblast-like cells (hOBs) from bone explant and their precursors from bone marrow (BMMs) and peripheral blood have also been extensively applied to study bone biology⁵⁷⁶. In comparison with hOBs cultures, osteoblastic lines are suitable *in vitro* pilot models for studying cell adhesion, regulation and production of OCN, but not for replacing primary cells to study cell proliferation and ALP activity owing to their different stages of maturation and resulting heterogeneous labelling profile with regard to the expression of osteoblastic markers^{577,578}.

In view of the emerging evidence on the importance of the immunomodulatory capacity of advanced bone biomaterials, the role of immune cells to regulate bone dynamic during the process of wound healing has recently been investigated^{579,580}. With respect to osseointegration, as previously mentioned, a number of studies have evaluated the impact of implant material and surface properties to activate several components of the innate immune response

following surgical placement¹³² and to regulate the inflammatory response in *in vitro* models²⁷⁵. For instance, the biochemical and molecular analysis of macrophages is possible through isolation of primary cells from murine and human bone marrow (BMMs) and peripheral blood or applying the model of RAW 264.7 murine macrophage-like cell line⁵²⁸. Results have shown that implant properties influence cellular processes beyond the osteoblastic activity, which may further modulate osteoclastogenesis in a surface dependent manner.

Despite the wide-ranging application of *in vitro* analyses in oral implant research, there is no ideal model that fully represents the clinical parameters due to the absence of the three-dimensional microarchitecture of the bone, its metabolic functions and modulators, and the biological response orchestrated by cell-to-cell communication; although evaluation of individual signals is also of great importance.

Yet, cell culture assays comply with the principles of the 3Rs (Replacement, Reduction and Refinement) for the absolute or relative substitution of laboratory animals, or by applying the data to minimize their usage and optimize current experimental protocols. Thus, further evaluation in *in vivo* animal models prior to clinical testing must be supported by previous *in vitro* results, if applicable, where discrepancies are common to occur implying biological mechanisms that trigger differentiation and activation of immune cells during peri-implant bone healing remain unclear and warrant future investigation.

***In vivo* models to study tissue response and implant integration**

According to The American National Research Council Committee on Animal Models for Research and Aging: “An animal model for biomedical research is one in which normative biology or behavior can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans or other species of animals”. s

Although there is no specific animal model that resembles the highly variable human pathophysiological mechanism, cranio-

maxillofacial anatomical, biomechanical and functional properties⁵⁸¹, preclinical experimentation is necessary for analysis of the biological and tissue response of novel biomaterials as preliminary evidence of biocompatibility and efficacy prior to clinical research⁵⁸². Therefore, an appreciation of their physiological and anatomical aspects (*e.g.*, bone metabolic rate, mineral density, and site dimensions) contribute to interpretation of significant data.

Accordingly, different species are suitable for different stages of preclinical testing of biomaterials, each one of them presenting several advantages and limitations⁵⁸³. The key aspect to be considered in any preclinical *in vivo* study, whether performed in small or large animals, is to what extent differences between the model and the human situation may affect clinical translational research. The limited application of various surgical locations is based on the fact that the quality of bone differs between anatomical sites and even within a single limb⁵⁸⁴, from mesial to distal sites, substantially leading to distinct tissue regeneration and integration from that found intra-orally in humans⁷⁷.

For instance, small animals, such as mice, rats, and rabbits, are considered superior models for initial screening of material biocompatibility, cytotoxicity, and potential adverse reactions than skeletally large animals, for being easier to handle and house, and for more convenient ethical requirements⁵⁸¹. They also facilitate the evaluation of a large sample size with sufficient power for statistical analysis in addition to an increased cost-effectiveness.

The use of microbiologically and genetically defined small animals result in better data reliability and reproducibility, and greater resistance to infection and diseases; in spite of greater risk of morbidity compared to large animals. Moreover, small-animal models are better candidates for chemically/surgically or genetically induced metabolic syndromes (*e.g.*, osteoporosis and diabetes) or applying radiotherapy to investigate fundamental mechanisms that affect biomaterial integration⁵⁸⁵.

Notwithstanding, mice and rats present the greatest variation in bone metabolism, remodeling, and anatomy compared to humans. Thus, international standards established rabbits, dogs, minipigs, sheep, and goats as the most suitable species for testing the efficacy of the final biomaterial designs and/or further optimization of

commercially available implantable devices⁵⁸⁶, including oral implants^{581,587,588}. Rabbits show the least bone remodeling period (6 weeks), in addition to a primary bone structure, whilst large animals, such as dogs and minipigs, present a greater similarity (approximately 3 months) compared to humans (3 - 6 months)⁵⁸⁶.

Still, the use of large animals, in special companion animals and non-human primates, raise additional ethical concerns and integrity-based assumptions based on the adverse effects of procedures and how they impact their welfare⁵⁸⁹. Thus, experimentation in these animals should be limited to the evaluation of biomaterials properties that warrant further proof-of-principle (*e.g.*, pilot studies), optimization and improvement of clinical efficacy upon a legal regulatory framework.

On the other hand, a higher number of experimental sites and units are possible to be applied in both intra- and extra-oral platforms of large animals, reducing the number of subjects needed for significant comparisons and, therefore, supporting the principles of the 3Rs. In the former situation, the split-mouth design is a common strategy in which each of the experimental and control groups is randomly assigned to either the right or left segments of the jaw, contributing to reduce the inter-individual variability⁵⁹⁰.

In the current thesis, the dog tibia (study I and II) and minipig mandible (study V) were selected to test the histomorphometrical parameters and biomechanical properties of commercially available implants at early and late stages of osseointegration.

Dogs of different strains, small (Beagle) and larger breeds (Mongrel, Labradors, German sheepdogs, etc.), or mixed breeds, have been extensively valuable in basic research on dental implants designs, materials, and surgical techniques. However, there is a limited application of different surgical locations due to distinguished bone types. The mandible of dogs is the most frequent testing site in studies evaluating implant integration, however, there is a high risk to the development of peri-implantitis due to the accumulation of oral biofilm upon negligent hygienic procedures⁵⁹¹.

As an alternative, a high percentage of studies on implant integration have used extra-oral implant sites. The tibia, for instance, due to its anatomy with a large lumen and relatively low bone density, offers better discriminating capacities than the rich in trabecu-

lar bone mandible for evaluating the potential of surfaces to significantly enhance osseointegration; in addition to requiring a more simplified surgical procedure and reduced morbidity^{272,592}. Furthermore, the tibia allows placement of a larger number of implants comparing with the mandible, thus allowing the use of fewer animals and/or multiple types of comparisons/tests (*e.g.*, biomechanical and histological evaluation).

In turn, barrier-bred minipigs are suitable for applications in *in vivo* research due to well-controlled health conditions and full maturation of their cranio-maxillofacial anatomical structures⁵⁹³. They are often used as an animal model to evaluate the potential of implants to osseointegrate because of the comparable bone metabolic rate (ratio of 1:2) and heterodont dentition to that in humans⁵⁸⁸. Concerning desirable attributes of animal models to analyze potential effects of varied surgical techniques of the implantation bed, the edentulous alveolar crest of minipigs presents a nearly similar bone composition and density compared to humans with a rather thick cortical bone and moderate to dense trabeculae, and high biomechanical properties⁵⁹⁴.

Nonetheless, the generalizability of findings presented in study V is subject to certain limitations. The edentulous alveolar crest of minipigs present a rather thick cortical bone and moderate to dense trabeculae with high biomechanical properties, which might synergistically intensify the effect of lateral compression as less porous, non-elastic and poorly vascularized bone is more inclined towards microstructural stresses, compromising normal metabolic activity and tissue regeneration⁵⁹⁴. In addition, narrow residual alveolar ridges are frequently found, which represent a high risk of developing peri-implant bone dehiscence and bone resorption⁵⁹⁵.

The effect of hydrophilicity on osseointegration and biomechanical properties of titanium implants

The alternative hypotheses that implants with a sandblasted and dual acid-etched surface (SAE), specifically treated to increase hydrophilicity (SAE-HD), would achieve higher BIC and interfacial shear strength, expressed as higher maximum RTV, removal energy, and connection stiffness, in comparison to SAE implants were tested in study I and II in a model of dog tibia.

Biological aspects

In study I, the results have shown that SAE-HD implants achieved similar amounts of BIC and peri-implant bone density as SAE, during the early stages of healing (2 and 4 weeks). These findings are in contrast with the results of most of the previously published pre-clinical *in vivo*^{295,366,596–598} and clinical studies^{123,599} evaluating hydrophilic implant surfaces. In those studies, implants with chemically modified hydrophilic surface – but different macrodesign and microtopography than the implants used herein – showed improved performance over relevant control implants.

For example, in an experiment conducted by Buser et al. (2004)²⁹⁵, where hydrophilic moderately rough implants (SLActive) were inserted in the maxilla of miniature pigs and compared with similar SLA implants – but without the specific surface treatment to increase hydrophilicity – higher amounts of BIC were observed at the hydrophilic implants after 2, 4, and 8 weeks of healing. Similarly, in another study in dogs, SLActive implants showed greater amount of newly formed bone in contact with the implant in comparison with the conventional SLA after 2 weeks of healing; however, after 8 weeks of healing similar amounts of BIC were observed at both types of implants⁵⁹⁶.

Recently, Wennerberg et al. (2013)³⁵¹ validated the structural formation of nanoparticles on SLActive surfaces manufactured in a similar way as to SAE-HD herein, exhibiting physical characteristics similar to the cellular microenvironment. As mentioned earlier, increased surface wettability aims to increase the implant surface area which favors protein adsorption and cellular adhesion, and thereby positively regulates the biological response at the initial osseointegration process (see the subsection “physical and chemical properties”). Hydrophilic surface modification has been shown to achieve rapid coverage of the implant surface with blood, enhance clot formation, upregulate expression of various angiogenic factors^{353–355}, and activate the production of anti-inflammatory factors and downregulate the expression of pro-inflammatory cytokines^{135,275,294,356,357}, which positively regulates osteogenic differentiation and maturation of osteogenic precursors cells^{257,294,358,359}.

In a study in rabbits, a new commercially available dental implant (Acqua[™], Neodent[®]; $\theta < 5$) manufactured by an almost iden-

tical technique as the SAE-HD herein was shown to possess a significantly higher hydrophilicity compared to a commercially available implant with surface technology identical with the SAE implant (NeoPoros®, Neodent®; $\theta > 90^\circ$)^{312,600}. Furthermore, “hydrophilic” implants placed in the tibia of the rabbits achieved 1.5 times more BIC compared with the “hydrophobic” ones after 28 days of healing³¹². Similarly, a recent study Wennerberg et al. (2014)²⁴⁷ applying the same animal model, showed that osseointegration, as assessed by pull-out force, was significantly enhanced by nanostructured hydrophilic implants (SLActive) after 4 and 8 weeks compared to SLA.

Another study evaluating Acqua implants, performed in a model of sheep tibia, has shown that small-diameter implants composed of Ti6Al5V-ELI alloy (grade 23) with a different thread geometry than the one used herein, resulted in significantly greater BIC and bone density at 2, 3, and 4 weeks of healing compared to NeoPoros®⁶⁰⁰. The same phenomenon was observed when applying short cpTi Acqua implants with, however, similar macrogeometry, indicating the higher potential of this hydrophilic surface to accelerate the healing period hydrophobic surfaces⁶⁰¹.

Comparison of BIC values among studies evaluating different hydrophilic technologies should of course be made with care, unless the studies include direct evaluation between specific surface technologies; surface chemistry at implants can indeed be easily changed by altering surface roughness⁶⁰². Also, differences in BIC values among studies may relate to differences in the experimental model used, regarding – among other factors – the species, bone density in the site of implantation, healing time, and method for histomorphometric estimation. In perspective, lack of significant differences between the SAE-HD and SAE implants in the present study seems unlikely to be due to lack of significant hydrophilicity of this specific manufacturing technology, although this possibility cannot exclusively be rejected.

In a recent systematic review of human histological studies on molecular aspects of early stages of osseointegration⁶⁰³, it has been confirmed that chemical modifications of moderately rough implant surfaces resulting in increased hydrophilicity, indeed seem to enhance molecular processes related to osseointegration during the

early stages of wound healing in the clinic. For example, specifically designed titanium devices with SLActive surface, implanted in the retromolar area in humans, achieved significantly higher amounts of BIC after 2 and 4 weeks comparing to devices with SLA surface; after 6 weeks, however, these differences were no longer evident⁵⁹⁹. Thus, a possible explanation for the lack of significant differences between the two groups herein could be the fact that the effect of hydrophilicity was unfolded during the very early periods of wound healing, *i.e.*, before the first evaluation time-point of 2 weeks in the present study.

Indeed, in a recent study reported by Vasak et al. (2015)³⁶⁶, hydrophilic implants (SLActive) strengthened the apposition of newly formed bone at the very early healing period between 5 and 10 days in the intra-oral model of minipigs, even though they did not reveal significant statistical differences in comparison to hydrophobic surfaces (SLA).

As mentioned above, studies providing histological evidence have reported that the advantage of hydrophilic implants was possible to observe only during the early stages of osseointegration, but “disappeared” at later stages, with control implants achieving similar amounts of BIC.^{596,599} In fact, studies using the same animal model (tibia of dogs), did not see any differences in terms of osseointegration of titanium implants with different surface technologies after healing times of 2 and 4 weeks^{522,604}.

Furthermore, Acqua implants have shown increased BIC and RTV following 15 and 45 days after placement in grafted areas in a model of rat tibia, with significantly higher expression of BMP-2 at the latter healing period compared to machined implants, irrespective of the grafting biomaterial³⁶⁴.

Biomechanical aspects

In study II, both SAE-HD and SAE implants showed relatively high RTV at both observation times, with the former group presenting about 100% higher values in removal energy. Moreover, SAE-HD implants showed high values in connection stiffness already after 2 weeks of healing, while SAE implants required 4 weeks of healing to reach a similar level. Despite absence of statistically significant differences for any of the evaluated parameters of observation

times, the results obtained herein indicate that differences in surface properties between SAE-HD and SAE implants, somehow influenced osseointegration and intrinsic properties of shear strength.

Indeed, greater RTV and interfacial stiffness for hydrophilic implants (modSLA) between 2 and 4 weeks, in comparison with SLA, have been previously reported²⁹⁶. In this study, performed in the anterior maxilla of miniature pigs, hydrophilic implants revealed, on average, 8 - 21% and 9 - 14% significantly higher RTV and interfacial stiffness, respectively, than those of the SLA® implants. Due to the remodeling process, the biomechanical parameters decreased with time for both implant surfaces, reflecting the developing biological stability.

It has been previously reported the existence of a correlation between RTV and BIC³⁶⁵, although the nature of these parameters differs from one another (3D versus most often 2D parameter)⁶⁰⁴. Indeed, the lack of differences between the groups herein reflect well the results of the histomorphometric analysis.

In fact, the observed lack of differences between the groups could be attributed to the relatively low number of specimens per group. In context, based on the observed results in the SAE group, only a rather large difference in BIC (37%) and RTV (43%) between the two groups would have been possible to disclose with enough power (80%) and the current sample size.

The potential of hydrophilicity to positively modulate the early inflammatory response and its effect on osteoclast differentiation, activation, and survival

In study III, the aim was to evaluate the influence of the implant thread design and surface hydrophilicity on osteoclast (OC) differentiation, activation, and survival *in vitro* in a model of RAW 264.7 cells.

Results obtained in this study show that implant surface hydrophilicity modulate osteoclast viability in the model of RANKL-induced osteoclastogenesis *in vitro*. Qualitative and quantitative assessment of cell morphology and adhesion revealed fundamental differences in a substrate-dependent manner. The total number of attached Mo/Mφ and OCs on hydrophilic surfaces was decreased compared to hydrophobic surfaces. SAE group was shown to facili-

tate cellular adhesion to the surface, which may have contributed to higher cellular viability, further confirmed by the metabolic activity of MTT.

Fundamentally, attachment of $\alpha_v\beta_3$ integrin and cytoskeletal rearrangement is mediated by actin rings resulting in cellular spreading (podosome clusters), motility (podosome rings), and sealing zone (SZ) assembly (actin belt)⁶⁰⁵. Integrin-based podosomes and actin rings, characterized in pre-mature and mature osteoclasts, respectively⁵⁵³, were identified on all substrates.

Recent *in vitro* studies have shown that topographical modifications of biomaterials affect the assembly of the sealing zone (SZ) and resorption apparatus (RA). Particularly, surface roughness at the micro and nanoscale level was shown to limit SZ expansion throughout ridge-like barriers and interfering with RA formation^{606,607}. These observations support a limited and short-term formation of actin rings on structured micro to nano-roughened substrates.

Conversely, the osteoclastogenic response was equally increased by rough ($R_a > 2 \mu\text{m}$) and moderately-rough surfaces ($R_a = 1\text{-}2 \mu\text{m}$) as it occurred on bone, while cellular differentiation was considerably decreased on smooth substrates displaying nearly absent actin rings⁵²⁶. Nevertheless, when compared to SLA, moderately rough hydrophilic surfaces (modSLA) were shown to negatively modulate osteoclast differentiation by decreasing attachment of monocytes in an osteoclastogenesis-induced model of murine bone-marrow derived macrophages (BMMs)³⁶², similarly to the present findings.

OC differentiation was assessed by means of enzymatic TRAP activity. The present findings confirm that enzymatic TRAP activity of cells grown and differentiated on HLX/SAE-HD, HLX/SAE, and TMX-SAE-HD surfaces was significantly lower than on TMX/SAE surfaces and CCPC (+). However, no significant differences were detected at the mRNA level of gene expression encoding this enzyme.

Even though increasing synthesis of this acid phosphatase is an indicator of osteoclast formation and bone resorptive activity, it is also highly expressed in fused and activated macrophages in pathological states, playing a critical role in innate immune response; further, recent findings have shown TRAP expression in osteocytes

and osteoblasts in close proximity to intracortical remodeling sites and cancellous bone surface⁶⁰⁸. Thus, increased enzymatic TRAP activity may be related to the inflammatory status mediated by macrophages under the influence of different implant surface modifications, not necessarily indicating osteoclast differentiation and activation in the present *in vitro* conditions.

Consistently, Brinkmann et al. (2012)⁵²⁶ reported significant higher TRAP activity on bone compared to those grown on Ti surfaces. Considering that translation and protein abundance depend on cytoskeleton rearrangement, the main reason behind this finding is the presence of biochemical signals on bone, but not on biomaterials. Hence, lack of significant differences at TRAP-related mRNA levels of gene expression on Ti disks may be explained by the fact that functional membrane structures, such as SZ, RA, and functional secretory domain (FSD), are not entirely generated by mature OCs when cultured *in vitro*, but only when in contact with osseous matrix (*i.e.*, phenotypic change of gene expression by environmental influence)⁶⁰⁹.

Arguably, titanium implants may inhibit osteoclast polarization and vesicular trafficking when in contact with the surface throughout the formation of podosome clusters, rings, or SZ-like podosome belts, rather than a stable sealing zone. These changes in the cellular phenotype may result in disparities between gene expression and post-translational modification of protein levels⁵²⁸. Furthermore, the correlation between mRNA and protein depends on other biological factors, such as cell cycle and its maturational stage, that may influence transcription levels, mRNA stability, translational rate, and protein turnover^{533,534}. Altogether, such a mechanism may explain the discrepancy in enzymatic TRAP activity and gene expression levels observed herein.

During bone resorption, additional proteolytic enzymes are secreted into the resorption lacunae, such as MMP-9 and CTSK, and result in respective mineral and organic bone matrix (type I collagen) cleavage and degradation¹⁹⁵. Further osteoclast activity-related gene expression revealed no significant effect of surface hydrophilicity and macrogeometry on bone resorption activity (CTSK, MMP-9, and CALCR), its negative regulator (ARG-1) nor on the expression of the pro-apoptotic gene BAX.

Although there were no significant differences in the gene expression of CALCR gene, its upregulation in the TMX/SAE-HD and TMX/SAE groups may imply stimulation of a more mature OC phenotype compared to the positive control group; or might be distinctively related to the aforementioned rate of high cellular proliferation and viability. In turn, ARG1 is a well-known biomarker of wound healing macrophage phenotype (M ϕ 2) and its expression is upregulated during phagocytosis, RANKL-induced osteoclast differentiation, and bone resorption; while its knockdown enhances formation of mature OCs²⁰⁴. In the present study, positive controls revealed a high level of ARG1 gene expression, suggesting the prevalence of M ϕ 2 under induced osteoclastogenesis on polystyrene *in-vitro*.

Gene expression levels of IL-6 and TNF- α were significantly upregulated in TMX/SAE-HD. This event might suggest a relationship between a low rate of osteoclast differentiation accompanied by cytokine production and promotion of pro- and/or anti-inflammatory microenvironment, suggesting the prevalence of pro-inflammatory macrophage phenotype (M ϕ 1) on moderately rough surfaces. In contrast, hydrophobic micro-rough implant surfaces have been shown to activate M ϕ 2 *in vitro* by upregulating the macrophage attractant chemokines (monocyte chemoattractant protein-1 [MCP-1] and macrophage inflammatory protein-1 alpha [MIP-1 α]) which stimulated a higher cellular proliferative rate⁶¹⁰.

In turn, hydrophilic surfaces were shown to further activate the highest production of anti-inflammatory factors and down-regulate the expression of key pro-inflammatory cytokines (*e.g.* IL-1 β , IL-6, and TNF- α) by osteoblasts³⁵⁶ and macrophage-like cells²⁷⁵. Regarding its biological relevance, IL-6 is a multifunctional cytokine that plays a central role in the anti-inflammatory/regenerative and pro-inflammatory responses, causing inhibition of regulatory lymphocytes (Treg), survival of T-lymphocytes and differentiation of Th17 cells⁶¹¹.

The effect of oversized surgical preparation of the implant site on osseointegration and marginal bone level

The effect of oversized surgical preparation of the implant site in terms of biomechanical (study IV) and biological parameters of os-

seointegration (study IV and V) was assessed in experimental animal models.

Specifically, in study IV, the current evidence on the effects of oversized surgical preparation of the implant site in terms of biomechanical and biological parameters of osseointegration in experimental animal models was systematically assessed and a meta-analysis of the estimate of efficacy of oversized vs. standard or undersized surgical preparation of the implant site was performed.

In study V, the effect of oversized surgical preparation of the implant site (OD, IDD = 0.1 mm in the 8-mm central apical length of the osteotomy and IDD = 0.0 mm in the 3.5-mm coronal length) on MBL and histomorphometrical parameters (BIC and BD) of tapered implants with a progressive thread geometry in the mandible of minipigs was evaluated following 12 weeks of non-submerged healing in the minipig mandible and compared to a standard-drilled site (SD, IDD = 0.3 mm). The alternative hypotheses that the oversized drilling technique, where healing chambers are initially formed due to sequential tapping and profiling of the bone in respect to the implant macrogeometry, would promote higher amount and better quality of osseointegration in comparison to the press-fit condition, was tested.

Biomechanical aspects

In regards with biomechanical outcomes, the meta-analysis performed in study IV showed that oversized surgical protocols (test OD and test GAP) resulted in significantly decreased values of maximum ITV and RTV compared to SD in extra-oral sites of large animals where the IDD ranged from 0.35 – 0.5 mm. In other words, ITV significantly decreases as a function of reducing the IDD, as confirmed in study V, where OD resulted in low ITV compared to SD.

Insertion torque is relative to the level of radial contact stress (*i.e.*, mechanical friction) and lateral compression applied to the surrounding bone during implant placement, which value changes according to the implant hardware design, *i.e.*, the interplay between implant macrogeometry and surgical preparation of the implant site, in association with the bone type (mineral density and cortical thickness).

For instance, implants placed in over-dimensioned sites do not exert frictional and compressive forces beyond the threshold of the bone owing to stepwise drilling instrumentation and passive surgical installation. Oversized implant sites generate low to nearly absent mechanical engagement of the implant threads within the bone^{401,500}, which is translated into low ITV (≤ 20 N.cm), particularly in poor-quality bone (type IV). The implant analyzed in study V presents a full dual tapered body with a dynamic progressive thread design varying between compressing trapezoidal design on the coronal area to self-tapping triangular threads on the apical part with different sizes. The use of an oversized drilling technique enables the formation of healing chambers of varied dimensions. Hence, the final drill employed with a 3.4 mm and 3.5 mm diameter for tapping of the bone site and profiling of the cortex, respectively, resulted in a 0.1 mm narrower implantation bed compared to the implant outer diameter plus a passive cortical layer (3.5 mm in length); *i.e.*, the threads engaged 0.05 mm within the trabecular bone, while there was a passive-fit at the coronal portion.

In contrast, implants installed in narrower sites prepared with standard (≥ 30 N.cm) or undersized drilling techniques (≥ 50 N.cm) yield high ITV, which stress concentration and deleterious effects are worsened in thick cortical bone and high-density trabeculae due to a low microstrain threshold and inelastic deformation (see the subsection “osteocompression and biomechanical strain”). In study V, standard drilling technique with a 3.2-mm final drill created a press-fit condition with the dimension of the implantation bed 0.3 mm smaller than the outer implant diameter, while the threads engaged 0.15 mm within the bone, including the cortical layer, where ITV ranged between 50 and 60 N.cm.

As aforementioned, despite the ever-growing scientific evidence on the clinical significance and complexity of insertion torque, high values may not be translated into corresponding high primary implant stability. In a study performed by Eom et al.³⁹⁸ and reported in study IV, undersized and standard drilling techniques, yielding high initial ISQ ($> 70\%$), maintain rather stable values up to 5 weeks of healing, in a similar animal model as in study V³⁹⁸. In turn, despite oversized drilling resulting in lower ISQ values ($\leq 60\%$) at the time of implant placement, a large increase in the total

implant stability threshold is observed already at the first week up to 3 weeks of healing, with minimal change until 6 weeks.

A recent preclinical study evaluating short implants in sheep mandible has demonstrated that an undersized-drill site (IDD = 0.3 mm) triggered major remodeling of the cortical bone associated with significantly lower RTV at 5 weeks of healing compared to a non-undersized site (IDD = 0.0 mm)⁵¹¹. Overall, after 10 weeks of healing, studies show comparable high levels of secondary implant stability irrespective of the drilling and implant macrodesign^{511,612}.

In spite of limited evidence, these observations of gradual increase of total implant stability in oversized implant sites reflect the events of intramembranous ossification in the presence of healing chambers, which contrasts the type of healing mode during interfacial remodeling in press-fit situations, experienced as a transient primary implant stability dip after a few weeks of implantation.

Biological aspects

Although over-dimensioned implant sites generate lower primary stability compared to standard and undersized sites³⁹⁸, the available literature shows that rotational resistance is just sufficient to allow a mechanically stable environment that favors initial events of bone healing, such as angiogenesis, under unloaded conditions⁴⁸³.

In study IV, systematic analysis of the available literature suggested that oversized surgical technique prevents ischemic necrosis and extensive bone resorption at the bone-implant interface both in the marginal cortical layer and in the trabeculae compared to both standard and undersized preparation of the implant site. As a result, faster and increased bone formation is observed around implants placed in over-dimensioned sites, mainly characterized by primary osteons and highly vascularized tissue following 1 week up to 5 weeks of healing.

Similarly, in study V, despite no significant differences in marginal bone levels after 12 weeks *in vivo*, histomorphometrically evaluated by means of f-BIC, there was a tendency for lesser bone resorption in over-dimensioned sites with profiling of the crestal bone compared to standard sites without profiling. In the latter group, overcompression and high interfacial stress concentration in highly dense and poorly vascularized cortical bone appeared to

cause greater crestal bone resorption associated with crater formation and impaired osseointegration as a result of potential microdamage and/or compression necrosis^{613–615}.

Excessive bone resorption following the first weeks upon implant placement results in low new bone appositional rate (2 – 5 $\mu\text{m/day}$)³⁷⁹, which has been shown to negatively affect its microstructural composition, cortical porosity, and biomechanical competence throughout the healing period in *in vivo* studies^{170,386,511}. For instance, Duyck et al. (2010)¹⁷⁰ reported significantly higher 4-mm CBL around implants inserted in undersized-drilled sites compared to oversized sites with profiling of the cortex (IDD = 0.4 and 0.0 mm, respectively).

In a study performed in the mandible of sheep, Stocchero et al. have demonstrated that a 0.3 mm-undersized implant site triggered major remodeling of the cortical bone associated with significantly lower new BIC and bone density at 5 weeks of healing compared to a non-undersized site (IDD = 0.0 mm). Clinically, a multicenter retrospective study showed that undersized sites (IDD = 0.5 mm) with only tapping of thick cortex of high mineral density) provoked significantly greater CBL in comparison to non-undersized sites receiving profiling (IDD = 0 – 0.15 mm) and tapping (IDD = 0.15 mm), after an average of 6 months⁵⁰³.

In contrast, studies using similar animal models as in study V have not found any statistically significant differences in CBL. A study evaluating oversized and undersized implant sites (IDD = 0.0 and 0.2 mm, respectively), in the minipig mandible, has reported comparable CBL of 0.5 mm after 5 weeks *in vivo*, despite the occurrence of microcracks in the cortical bone in the latter group³⁹⁸.

At the long-term healing of 12 weeks, similar values were also observed in sites receiving only tapping against oversized sites with profiling of the cortex (IDD = 0.15 and -0.05 mm, respectively)⁵¹⁵. Similarly, a recent meta-analysis observed similar CBL between high and low ITV in both human and animal studies at the long-term follow-up⁶¹⁶. However, deleterious effects of bone overcompression and higher stress concentration in the cortical layer appears to be worsened under immediate functional loading, leading to the formation of crater-like bone defects⁶¹⁷ and even impaired osseointegration⁵¹⁵.

Notwithstanding, studies reporting on MBL under the influence of various surgical drilling protocols under functional loading are limited and do not allow further assumptions. In a study performed in the dog mandible, Rea et al.⁶¹⁸ showed that implants placed in oversized sites (IDD = 0.0 mm; ITV = 30 N.cm) and immediately loaded, revealed lower marginal resorption of the buccal bone compared to implants placed in undersized sites (IDD = 0.3 mm; ITV > 70 mm) under unloaded conditions. However, at the long-term clinical follow-up, there is no difference in MBL between high and low ITV⁶¹⁶.

Regarding biological parameters of osseointegration, the meta-analysis performed in study IV showed that test OD resulted in overall reduced BIC in the early period of healing, while statistically similar values were revealed at 3 weeks compared to SD (IDD = 0.2 – 0.55 mm). Additionally, test OD showed significantly reduced relative bone density up to 2 weeks and, again, following 5 weeks of healing – with similar amounts between 3 and 4 weeks compared to SD. Meanwhile, during the latter period, test GAP resulted in reduced values of bone density compared to SD (IDD = 0.2 – 0.4 mm), suggesting that the presence of a gap between the implant and the bone wall appears to not outperform the amount of bone formation observed in press-fit situations.

Nevertheless, in study V, oversized sites resulted in significantly higher BIC and peri-implant bone density, characterized by osteonic structures within the threads after 12 weeks *in vivo*. Healing chambers filled with blood clot have been shown to modulate cellular responses during the early process of tissue repair by creating an osteoprotective microenvironment that supports the bioactivity of implant surfaces, the latter acting as implant hardware adhesions⁶¹⁹.

As a result of tension release in the absence of microfractures, healing chambers support proliferative mechanisms during osseointegration without prior resorption of damaged bone^{152,398,401}. In this scenario, multidirectional bone growth occurs on the implant surface and from the surgically instrumented pristine bone walls towards the central region of the chamber throughout an intramembranous-like healing pathway³⁷⁹. In several studies, void spaces were shown to be rapidly filled with WB presenting high

degrees of vascularization and cellular composition already in the first week upon implant placement with subsequent maturation surrounding the perimeter of primary osteons^{384,398,401,483,500,502}.

Similar microstructures have been previously found in healing chambers shaped around plateau implants as early as 2 and 5 weeks *in vivo* in association with multiple modeling sites, where the bone formation rate varied between 30 and 50 μm per day³⁷⁹. Accordingly, fast bone matrix apposition and mineralization in healing chambers may develop similar amount of bone density and implant stability following few weeks after implantation compared to those found in implants that undergo interfacial bone remodeling in undersized sites. Thus, oversized sites might contribute to strength bone mechanical properties throughout the formation of a multidirectional Harversian-like channel system, and thereby may promptly adapt to functional loading forces^{375,376,484}.

These histological findings strongly indicate that oversized-drilled sites prevent early marginal bone loss along with an increase in the rate of BIC and bone density as time elapses. In turn, press-fit conditions in standard- and undersized-drilled sites result in primary bone remodeling prior to osteogenic tissue apposition, especially between 2- and 4-weeks post-operatively, potentially delaying or impairing osseointegration.

Noteworthy is that implant body shape and thread design influence the degree of lateral mechanical compression and stress distribution at the bone-implant interface upon surgical placement^{432,620}. In study IV, some studies have suggested the benefit of hybrid healing pathway to enhance the performance of implants following placement of implants with a dynamic progressive thread profile in over-dimensioned sites by integrating both interfacial remodeling and intramembranous-like healing modes^{369,501,523}. As in study V, this implant hardware design, associated with a tapered body, allows the tip of triangular self-tapping threads to slightly engage into the bone surrounding the implant apex, while square and trapezoidal threads in the central coronal portion of the implant form healing chambers. Such a scenario attains proper levels of primary implant stability while preventing compressive strain, especially in thick crestal bone and highly dense trabeculae.

Rapid woven bone formation and ossification in healing chambers compensate the potential loss of primary stability that will follow the events of interfacial remodeling in sites initially in direct contact to the implant surface, hastening osseointegration and enhancing the load-bearing capacity of implants placed already in the early healing period compared to press-fit situations⁶²¹. However, depending on the status of the host bone and loading condition, mechanical resistance against micromotion in over-dimensioned sites might reach similar levels compared to those instrumented with standard and/or undersized drilling techniques during the early post-operative period.

There are several possible explanations to the lack of corresponding data to the findings between study IV and study V, such as the application of different animal models and, consequently, varied alveolar contour, bone quality, and cortical thickness, in addition to modifications in implant surface properties and macro-geometry. Likewise, the great variability in study design and quality of methodological analysis of the reviewed articles in study IV may have an effect in the calculation of pooled estimates and interpretation should be performed with care.

In study V, implants with a hydrophilic surface, proven to positively modulate the early cellular response³⁶³ and accelerate bone formation^{312,600}, were used. The majority of the studies evaluating oversized surgical protocols apply moderately rough surfaces with another chemical modification than that applied herein, possibly revealing different levels of surface energy, wettability, and chemical composition.

CONCLUSIONS AND FUTURE PERSPECTIVES

*Science is not only a disciple of reason, but, also,
one of romance and passion*

Stephen Hawking (1942 - 2018), English theoretical physicist

According to the results obtained in the present thesis, the following conclusions on aspects of oral implant technology and osseointegration were established:

1. Histomorphometrical parameters (bone-to-implant contact [BIC] and relative peri-implant bone density [BD]) increased with time at implants with a sandblasted, dual acid-etching, with or without an additional chemical modification (hydrophilic SAE-HD and hydrophobic SAE, respectively), and no significant differences were observed between the two groups for any of the evaluated parameters and at any observation time-point during the early osseointegration period in the tibia of Beagle dogs.
2. In terms of biomechanical properties (removal torque value [RTV] and a complete set of intrinsic properties [removal energy and connection stiffness]), no significant differences were observed between SAE-HD and SAE implants for any of the evaluated parameters and at any observation time-point but a slight increase over time during the early osseointegration period in the tibia of Beagle dogs.

3. Hydrophilic SAE-HD surfaces negatively modulate macrophage/osteoclast viability. Specifically, SAE-HD with triangular threads increases the cellular pro-inflammatory status, while surface hydrophilicity and macrodesign do not seem to have a distinct impact on OC differentiation and activation *in vitro* in a model of RAW 264.7 cells.
4. Oversized surgical preparation of the implant site appears to minimize marginal bone resorption characterized by fast rates of new bone formation in the healing chambers through an intramembranous-type healing mode. However, in terms of biomechanical properties and histomorphometrical parameters of osseointegration, oversized implant sites does not seem to provide any additional benefit compared to narrower implant sites, neither at the time of implant placement nor during the early healing period, yielding comparable results at the late post-operative period in different experimental animal models.
5. Oversized surgical preparation of the implant site yielded low insertion torque of tapered implants with a progressive thread geometry, but enhanced osseointegration and peri-implant bone density, and preserved better the MBL following 12 weeks *in vivo* in the mandible of minipigs.

In spite of the present thesis confirming the potential of the specific surface technology with increased hydrophilicity to promote successful osseointegration in *in vivo* models of large animals, there still remains a question concerning its effect on the modulation of the molecular and cellular mechanisms underlying the immune-inflammatory response and subsequent osteoclast-derived activity in the coupling mechanism during the early period of bone healing. This matter has grown in importance in light of recent evidence indicating that implant hardware design dictates the type of healing pathway that will follow the surgical procedure since surface properties will accordingly perform as the implant hardware ad-hocs. Hence, *in vitro* testing with co-culture including well-designed methodological strategies are warranted to explore aspects of osteoimmunology prior to performing *in vivo* experiments.

Furthermore, despite high clinical success rates of implants with significant hydrophilicity, incorporation of innovative technologies through functionalization with biochemical nanocoating to locally release chemical compounds, mineral ions, and drugs, offer a potential to further develop enhance surface bioactivity and accelerate osseointegration. Overall, these systems have shown to positively regulate osteogenic differentiation and bone apposition to the surface in a variety of preclinical studies, however, their clinical efficacy remains to be evaluated.

With regards to the interplay between implant macrogeometry and surgical drilling technique/dimension of the osteotomy, defined herein as implant hardware design, the present thesis has shown that oversized implant sites maintain marginal bone levels and support proper osseointegration in the minipig mandible, with major benefits in high-density bone presenting a thick cortical layer. Nevertheless, the current evidence on the effect of overdimensioned osteotomy in compromised sites (*e.g.*, reduced bone volume, immediate implant placement, and grafted areas) as well as in experimental settings of systemic disorders (*e.g.*, osteoporosis), is limited and warrant further investigation in *in vivo* models and controlled clinical trials. Moreover, studies evaluating the effect of immediate and early functional loading of implants placed in different osteotomy dimensions based on bone density and cortical thickness are limited and demand additional elucidation.

Finally, adequate patient selection and treatment planning are essential to guarantee high predictability and long-term maintenance of the peri-implant marginal bone. Thus, the results of this thesis are expected to contribute to a better understanding of hierarchical aspects that significantly impact the rate and quality of osseointegration, implant survival and success of different treatment modalities, inspiring future research and advances in oral implant technologies, and a better clinical decision making based on a systematic database.

POPULÄRTVETENSKAPLIG SAMMANFATTNING

Behandling med käkbensförankrade tandimplantat är en vanlig metod för att ersätta förlorade tänder hos helt och partiellt tandlösa patienter. Trots att implantatbehandling uppvisar en långsiktigt hög lyckande- och överlevnadsfrekvens (90–95% efter 10 år) så förekommer implantatförluster och marginal benförlust kring implantaten under det första året efter insättning och/eller belastning, vilket kan försämra det estetiska resultatet.

För att förhindra tidiga implantatförluster och/eller den marginala benremodelleringen görs olika modifieringar av de topografiska, fysikaliska och kemiska egenskaperna hos implantatytorna i syfte att påskynda och öka benbildningen i kontakt med implantatet (bone-to-implant contact [BIC]). Till exempel uppvisar en hydrofil yta med moderat råhet (höjdavvikelser på omkring 1.5 μm) en snabbare beläggning av blod jämfört med en hydrofob yta, vilket i sin tur påverkar den tidiga läkningen genom migrering och differentiering av benbildande osteoblaster. En annan metod som föreslagits för att förbättra osseointegrationen (beninläkningen) och den marginala bennivån kring implantaten är att öka primärtstabiliteten, d.v.s. den initiala mekaniska fixeringen i käkbenet. Implantatets primärtstabilitet påverkas av bentätheten, implantatets design (d.v.s. utformning och gängdesign) och det använda borrhprotokollet vid preparation av implantatsätet. Standardborrprotokollen innebär att ostetomien (borrhålet) är mindre än implantatets diameter ("relative implant-final drill discrepancy" [IDD]; 0.2 – 0.5 mm). Studier har visat att en underdimensionerad osteotomi, d.v.s. (IDD > 0.5 mm), kan förbättra osseointegrationen. Samtidigt har över-

driven kompression av marginalt ben med hög täthet visat sig orsaka marginal bennekros, vilket ökar benresorptionen och risken för tidig implantatförlust. I gengäld har "överdimensionering" av osteotomien, antingen med exakt samma dimension som implantatet eller med $IDD \leq -0.1$ mm större än implantatet uppvisat kontroversiella resultat.

Det primära syftet med avhandlingen var att utvärdera olika aspekter av nuvarande oralimplantatteknologi och dess effekter på osseointegrationen.

I delarbete I och II utvärderades hos djur den biologiska responsen (BIC och relativ bendensitet [BD], studie I) och de biomekaniska egenskaperna (maximala momentvärdena vid implantatsättning [RTV], borttagningsenergi och implantatanslutningsstyvhet, studie II) på. Resultaten visade att BIC, BD, RTV och borttagningsenergin ökade med tiden i både hydrofoba- och hydrofila-tytor. Emellertid observerades inga signifikanta skillnader mellan de två grupperna avseende de utvärderade parametrarna vid någon av observationstidpunkterna.

I delarbete III utvärderades påverkan av implantatsgängans design och ytvetbarhet på osteoklasternas differentiering, aktivering och överlevnad *in vitro*. Titandiskar framställdes med hydrofoba- och hydrofila-tytor och med gängor bestående av kvadratiska, trapezoid- och stödgeometri (progressiv gänggeometri) eller dubbeltriangulära geometri. Med molekylära analyser mättes osteoklasternas differentiering och livskraft, vidhäftning och morfologi samt expressionsnivåer av olika relaterade gener. Hydrofila tytor modulerade negativt makrofag/osteoklast livskraft. Specifikt ökar hydrofila tytor med dubbel-triangulära gängdesign den cellulära proinflammatoriska responsen, medan hydrofoba tytor och implantatsgängans design inte verkar ha någon tydlig inverkan på osteoklasternas differentiering, aktivering eller överlevnad.

I delarbete IV utvärderades de biomekaniska och biologiska effekterna av överdimensionerad, standard och underdimensionerad osteotomi genom en systematisk litteraturöversikt. Utfallet av ITV, RTV, BIC och BD vid olika läkningsperioder testades statistiskt för att upptäcka signifikanta skillnader. Resultat från 12 studier tyder på att överdimensionerade osteotomi verkar minska den marginala benresorptionen, kännetecknad av snabbare och ökad benbildning

i de ihåliga utrymmena. Emellertid uppvisar överdimensionerade osteotomier inte vid någon observationstidpunkt några ytterligare fördelar av de mätta osseointegrationsparametrarna jämfört med standardtillvägagångssättet eller underdimensionerade osteotomier.

I delarbete V utvärderades, hos djur, den biologiska responsen (BIC och relativ bendensitet [BD]) och MBL av överdimensionerad osteotomi (OD) på avsmalnande implantat med en progressiv gänggeometri i jämförelse med det standard tillvägagångssättet (SD) efter en läkningsperiod på 12 veckor. SD inkluderade ett borrhprotokoll med 3-stegsserie av borrar, medan OD omfattade en 5-stegsserie. Även om OD gav ett lågt ITV vid avsmalnande implantat med hydrofil yta så förbättrades osseointegrationen och bendensiteten och MBL bevarades bättre.

Sammanfattningsvis indikerar denna avhandling:

- Att hydrofila och hydrofoba implantat med en moderat yt-råhet uppvisar likartad potential för framgångsrik osseointegration med starka biomekaniska egenskaper.
- Att hydrofilitet modulerar negativ makrofag/osteoklast livskraft, men varken vätbarhet eller gängdesign verkar ha en distinkt påverkan på osteoklast differentiering, aktivering och överlevnad *in vitro*.
- Att överdimensionerad osteotomi uppvisar en stor heterogenitet och kontrovers när det gäller dess eventuellt positiva effekter på osseointegrationen jämfört med en standard osteotomi, men vid tillfällen av tätt och tjockt marginalt ben förbättrade överdimensionerade osteotomier osseointegrationen och bentätheten jämfört med standardtillvägagångssättet, samt bevarade bättre den marginala ben-nivån.

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*If I have seen further it is by standing
on the shoulders of Giants*

Sir Isaac Newton (1643 - 1727), English physicist

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REFERENCES

1. Cunha-Cruz J., Hujoel PP., Nadanovsky P. Secular trends in socio-economic disparities in edentulism: USA, 1972-2001. *J Dent Res* 2007. Doi: 10.1177/154405910708600205.
2. Marcenes W., Kassebaum NJ., Bernabé E., Flaxman A., Naghavi M., Lopez A., et al. Global burden of oral conditions in 1990-2010: A systematic analysis. *J Dent Res* 2013. Doi: 10.1177/0022034513490168.
3. Vos T., Abajobir AA., Abbafati C., Abbas KM., Abate KH., Abd-Allah F., et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 2017. Doi: 10.1016/S0140-6736(17)32154-2.
4. Emami E., De Souza RF., Kabawat M., Feine JS. The impact of edentulism on oral and general health. *Int J Dent* 2013. Doi: 10.1155/2013/498305.
5. Österberg T., Dey DK., Sundh V., Carlsson GE., Jansson JO., Mellström D. Edentulism associated with obesity: A study of four national surveys of 16 416 Swedes aged 55-84 years. *Acta Odontol Scand* 2010. Doi: 10.3109/00016357.2010.514721.
6. De Pablo P., Dietrich T., McAlindon TE. Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. *J Rheumatol* 2008. Doi: 07/13/1123 [pii].
7. LaMonte MJ., Genco RJ., Hovey KM., Wallace RB., Freudenheim JL., Michaud DS., et al. History of Periodontitis Diagnosis and Edentulism as Predictors of Cardiovascular Disease, Stroke, and Mortality in Postmenopausal Women. *J Am Hear Assoc Cardiovasc Cerebrovasc Dis* 2017;6(4). Doi: 10.1161/JAHA.116.004518.
8. Brodeur JM., Laurin D., Vallee R., Lachapelle D. Nutrient intake and gastrointestinal disorders related to masticatory performance in the edentulous elderly. *J Prosthet Dent* 1993.

Doi: 10.1016/0022-3913(93)90087-5.

9. Abnet CC., Qiao YL., Dawsey SM., Dong ZW., Taylor PR., Mark SD. Tooth loss is associated with increased risk of total death and death from upper gastrointestinal cancer, heart disease, and stroke in a Chinese population-based cohort. *Int J Epidemiol* 2005. Doi: 10.1007/s11356-018-3334-y.
10. Wang PY., Fang JC., Gao ZH., Zhang C., Xie SY. Higher intake of fruits, vegetables or their fiber reduces the risk of type 2 diabetes: A meta-analysis. *J Diabetes Investig* 2016. Doi: 10.1111/jdi.12376.
11. Ioannidou E., Swede H., Fares G., Himmelfarb J. Tooth Loss Strongly Associates With Malnutrition in Chronic Kidney Disease. *J Periodontol* 2014. Doi: 10.1902/jop.2013.130347.
12. Wiener R. Relationship of Edentulism, Sleep Disordered Breathing and Cardiovascular Disease: NHANES, 2007-2008. *Cardiol Angiol An Int J* 2015;3(4):167-74. Doi: 10.9734/CA/2015/17944.
13. Gerritsen AE., Allen PF., Witter DJ., Bronkhorst EM., Creugers NHJ. Tooth loss and oral health-related quality of life: A systematic review and meta-analysis. *Health Qual Life Outcomes* 2010. Doi: 10.1186/1477-7525-8-126.
14. MacEntee M., Glick N., Stolar E. Age, gender, dentures and oral mucosal disorders. *Oral Dis* 2008. Doi: 10.1111/j.1601-0825.1998.tb00252.x.
15. Jainkittivong A., Aneksuk V., Langlais RP. Oral mucosal conditions in elderly dental patients. *Oral Dis* 2002. Doi: 10.1034/j.1601-0825.2002.01789.x.
16. Jainkittivong A., Aneksuk V., Langlais RP. Oral mucosal lesions in denture wearers. *Gerodontology* 2010. Doi: 10.1111/j.1741-2358.2009.00289.x.
17. Adell R., Lekholm U., Rockler B., Branemark PI. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. *Int J Oral Surg* 1981;10(6):387-416.
18. ten Bruggenkate CM., van der Kwast WA., Oosterbeek HS. Success criteria in oral implantology. A review of the literature. *Int J Oral Implantol* 1990;7(1):45-51.
19. Albrektsson T., Wennerberg A. Oral implant surfaces: Part 1--review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. *Int J Prosthodont* 2004;17(5):536-43.
20. Balshi SF., Wolfinger GJ., Balshi TJ. A prospective study of immediate functional loading, following the teeth in a dayTM protocol: A case series of 55 consecutive edentulous maxillas. *Clin Implant Dent Relat Res* 2005. Doi: 10.1111/j.1708-

- 8208.2005.tb00043.x.
21. Meijer HJA., Raghoobar GM., Batenburg RHK., Vissink A. Mandibular overdentures supported by two brnemark, IMZ or ITI implants: A ten-year prospective randomized study. *J Clin Periodontol* 2009. Doi: 10.1111/j.1600-051X.2009.01442.x.
 22. Barewal RM., Stanford C., Weesner TC. A randomized controlled clinical trial comparing the effects of three loading protocols on dental implant stability. *Int J Oral Maxillofac Implants* 2012;**27**(4):945–56.
 23. Jimbo R., Albrektsson T. Long-term clinical success of minimally and moderately rough oral implants: A review of 71 studies with 5 years or more of follow-up. *Implant Dent* 2015. Doi: 10.1097/ID.0000000000000205.
 24. Meredith N. Assessment of implant stability as a prognostic determinant. *Int J Prosthodont* 1998;**11**(5):491–501.
 25. Albrektsson T., Branemark PI., Hansson HA., Lindstrom J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. *Acta Orthop Scand* 1981;**52**(2):155–70.
 26. Chrcanovic B. *On failure of oral implants*. Malm University, 2017.
 27. Williams DF. On the nature of biomaterials. *Biomaterials* 2009. Doi: 10.1016/j.biomaterials.2009.07.027.
 28. Ratner BD. Biomaterials Science: An Interdisciplinary Endeavor. *Biomaterials Science An Introduction to Materials in Medicine*. 2nd ed. Elsevier Academic Press; 2004. p. 1–8.
 29. Williams DF. There is no such thing as a biocompatible material. *Biomaterials* 2014. Doi: 10.1016/j.biomaterials.2014.08.035.
 30. Galli S., Stocchero M., Andersson M., Karlsson J., He W., Lilin T., et al. The effect of magnesium on early osseointegration in osteoporotic bone: a histological and gene expression investigation. *Osteoporos Int* 2017. Doi: 10.1007/s00198-017-4004-5.
 31. Offermanns V., Andersen OZ., Riede G., Sillassen M., Jeppesen CS., Almtft KP., et al. Effect of strontium surface-functionalized implants on early and late osseointegration: A histological, spectrometric and tomographic evaluation. *Acta Biomater* 2018. Doi: 10.1016/j.actbio.2018.01.049.
 32. Soares PBF., Moura CCG., Chinaglia CR., Zanutto ED., Zanetta-Barbosa D., Stavropoulos A. Effect of titanium surface functionalization with bioactive glass on osseointegration: An experimental study in dogs. *Clin Oral Implants Res* 2018. Doi:

- 10.1111/clr.13375.
33. Raphael J., Karlsson J., Galli S., Wennerberg A., Lindsay C., Haugh MG., et al. Engineered protein coatings to improve the osseointegration of dental and orthopaedic implants. *Biomaterials* 2016. Doi: 10.1016/j.biomaterials.2015.12.030.
34. Alenezi A., Naito Y., Terukina T., Prananingrum W., Jinno Y., Tagami T., et al. Controlled release of clarithromycin from PLGA microspheres enhances bone regeneration in rabbit calvaria defects. *J Biomed Mater Res - Part B Appl Biomater* 2018. Doi: 10.1002/jbm.b.33844.
35. Wennerberg A., Bougas K., Jimbo R., Albrektsson T. Implant coatings: new modalities for increased osseointegration. *Am J Dent* 2013;**26**(2):105–12.
36. Coelho PG., Teixeira HS., Marin C., Witek L., Tovar N., Janal MN., et al. The in vivo effect of P-15 coating on early osseointegration. *J Biomed Mater Res - Part B Appl Biomater* 2014. Doi: 10.1002/jbm.b.33020.
37. Smith DC. Dental implants: materials and design considerations. *Int J Prosthodont* 1993.
38. Brown SA., Lemons JE. *Medical applications of titanium and its alloys: the material and biological issues*. West Conshohocken, PA: ASTM; 1996.
39. J. E. *Nature's Building Blocks: An A-Z Guide to the Elements*. Oxford University Press; 2011.
40. Brånemark PI., Hansson BO., Adell R., Breine U., Lindström J., Hallén O., et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scand J Plast Reconstr Surg Suppl* 1977. Doi: 10.1007/s11064-007-9532-5.
41. Brånemark PI., Adell R., Breine U., Hansson BO., Lindström J., Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. *Scand J Plast Reconstr Surg* 1969;**3**(2):81–100.
42. Adell R., Hansson BO., Brånemark PI., Breine U. Intra-osseous anchorage of dental prostheses. II. Review of clinical approaches. *Scand J Plast Reconstr Surg* 1970;**4**(1):19–34.
43. Cotton FA., Wilkinson G., Murillo CA., Bochmann M. *Advanced inorganic chemistry*. 1999.
44. Zhao ZY., Li L., Bai PK., Jin Y., Wu LY., Li J., et al. The heat treatment influence on the microstructure and hardness of TC4 titanium alloy manufactured via selective laser melting. *Materials (Basel)* 2018. Doi: 10.3390/ma11081318.
45. Freese HL., Volas MG., Wood JR. Metallurgy and Technological Properties of Titanium and Titanium Alloys. In:

- Brunette DM, editor. *Titanium in Medicine. Engineering Materials*. Berlin/Heidelberg: Springer; 2001. p. 25.
46. Okazaki Y., Gotoh E. Comparison of metal release from various metallic biomaterials in vitro. *Biomaterials* 2005. Doi: 10.1016/j.biomaterials.2004.02.005.
 47. Anchieta RB., Baldassarri M., Guastaldi F., Tovar N., Janal MN., Gottlow J., et al. Mechanical property assessment of bone healing around a titanium-zirconium alloy dental implant. *Clin Implant Dent Relat Res* 2014. Doi: 10.1111/cid.12061.
 48. Crubzy E., Murail P., Girard L., Bernadou J-P. False teeth of the Roman world. *Nature* 1998. Doi: 10.1038/34067.
 49. Leventhal GS. Titanium, a metal for surgery. *J Bone Joint Surg Am* 1951. Doi: 10.2106/00004623-195133020-00021.
 50. Bothe RT., Beaton LE., Davenport HA. Reaction of Bone to Multiple Metallic Implants. *Surgery, Gynecol Obstet* 1940;**71**:598–602.
 51. Southam JC., Selwyn P. Structural changes around screws used in the treatment of fractured human mandibles. *Br J Oral Surg* 1971;**8**(3):211–21.
 52. Linkow LL., Weiss JL. The endosseous blade. A progress report. *Int J Orthod* 1969;**7**(4):155–65.
 53. Branemark PI. Vital microscopy of bone marrow in rabbit. *Scand J Clin Lab Invest* 1959.
 54. Branemark PI., Hansson BO., Adell R. Osseointegrated implants in the treatment of the edentulous. *Jaw Scand J Plast Reconstr Surg* 1977;**16 Suppl**:1–116.
 55. Branemark PI., Adell R., Albrektsson T., Lekholm U., Lundkvist S., Rockler B. Osseointegrated titanium fixtures in the treatment of edentulousness. *Biomaterials* 1983;**4**(1):25–8.
 56. Branemark PI. Introduction to osseointegration. *Tissue-Integrated Prostheses Osseointegration Clin Dent Quintessence Books* 1985:1–78.
 57. Zarb GA., Albrektsson T. Osseointegration: A Requiem for the Periodontal Ligament? *Int J Periodontol Restor Dent* 1981.
 58. Albrektsson T., Chrcanovic B., Jacobsson M., Wennerberg A. Osseointegration of Implants: A Biological and Clinical Review. *JSM Dent Surg* 2017;**2**(3).
 59. Jemt T., Chai J., Harnett J., Heath MR., Hutton JE., Johns RB., et al. A 5-year prospective multicenter follow-up report on overdentures supported by osseointegrated implants. *Int J Oral Maxillofac Implant* 1996. Doi: 10.1021/bi901074h.
 60. Lekholm U., Steenberghe D., Herrmann I., Bolender C., Folmer T., Gunne J., et al. Osseointegrated Implants in the Treatment

- of Partially Edentulous Jaws: A Prospective 5-Year Multicenter Study. *Int J Oral Maxillofac Implants* 1994;**9**(6):627–35.
61. Schnitman P. Immediate fixed interim prostheses supported by two-stage threaded implants: methodology and results. *J Oral ...* 1990. Doi: 10.1016/0921-4526(92)90560-F.
 62. Chiapasco M. Early and immediate restoration and loading of implants in completely edentulous patients. *Int J Oral Maxillofac Implants* 2004;**19 Suppl**:76–91.
 63. Schnitman PA., Wöhrle PS., Rubenstein JE., DaSilva JD., Wang N-H. Ten-Year Results for Brånemark Implants Immediately Loaded with Fixed Prostheses at Implant Placement. *Int J Oral Maxillofac Implant* 1997;**12**(4):495–503.
 64. Romanos GE., Aydin E., Locher K., Nentwig G-H. Immediate vs. delayed loading in the posterior mandible: a split-mouth study with up to 15 years of follow-up. *Clin Oral Implants Res* 2016;**27**(2):74. Doi: 10.1111/clr.12542.
 65. Chiapasco M., Gatti C. Implant-retained mandibular overdentures with immediate loading: A 3- to 8-year prospective study on 328 implants. *Clin Implant Dent Relat Res* 2003. Doi: 10.1111/j.1708-8208.2003.tb00179.x.
 66. Ma S., Tawse-Smith A., Brown SDK., Duncan W. Immediately restored single implants in the aesthetic zone of the maxilla using a novel design: 5-year results from a prospective single-arm clinical trial. *Clin Implant Dent Relat Res* 2019. Doi: 10.1111/cid.12733.
 67. Luongo G., Lenzi C., Raes F., Eccellente T., Ortolani M., Mangano C. Immediate functional loading of single implants: a 1-year interim report of a 5-year prospective multicentre study. *Eur J Oral Implantol* 2014;**7**(2):187–99.
 68. Amato F. Immediate functional loading of posterior implants placed in partially edentulous patients: a preliminary report on a prospective clinical study. *Int J Periodontics Restorative Dent* 2015;**35**(2):239–45.
 69. Pieri F., Aldini NN., Fini M., Marchetti C., Corinaldesi G. Immediate functional loading of dental implants supporting a bar-retained maxillary overdenture: preliminary 12-month results. *J Periodontol* 2009;**80**(11):1883–93. Doi: 10.1902/jop.2009.090227.
 70. Buckwalter JA., Glimcher MJ., Cooper RR., Recker R. Bone biology. I: Structure, blood supply, cells, matrix, and mineralization. *Instr Course Lect* 1996;**45**:371.
 71. Bilezikian JP., Raisz LG., Martin TJ. *Principles of bone biology*. Academic Press; 2008.
 72. Bloom W., Fawcett DW. *A textbook of histology*. Saunders;

- 1964.
73. Nanci A. *Ten Cate's Oral Histology Development, Structure, and Function*, 8/e. Elsevier India; 2012.
74. Eriksen EF., Axelrod DW., Melsen F. *Bone histomorphometry*. Raven Press; 1994.
75. Safadi FF., Barbe MF., Abdelmagid SM., Rico MC., Aswad RA., Litvin J., et al. Bone structure, development and bone biology. *Bone pathology*. Springer; 2009. p. 1–50.
76. Hauge EM., Qvesel D., Eriksen EF., Mosekilde L., Melsen F. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. *J Bone Miner Res* 2001;**16**(9):1575–82.
77. Lekholm U., Zarb GA. Patient selection and preparation. In: Branemark PI, Zarb GA, and Albrektsson T, editors. *Tissue integrated prostheses: osseointegration in clinical dentistry*. Chicago: Quintessence Publishing Company; 1985. p. 199.
78. Alghamdi HS. Methods to Improve Osseointegration of Dental Implants in Low Quality (Type-IV) Bone: An Overview. *J Funct Biomater* 2018;**9**(1). Doi: 10.3390/jfb9010007.
79. Marx RE., Garg AK. Bone structure, metabolism, and physiology: its impact on dental implantology. *Implant Dent* 1998;**7**(4):267–76.
80. Buck 2nd DW., Dumanian GA. Bone biology and physiology: Part I. The fundamentals. *Plast Reconstr Surg* 2012;**129**(6):1314–20. Doi: 10.1097/PRS.0b013e31824eca94 [doi].
81. Hogan BLM. Bone morphogenetic proteins in development. *Curr Opin Genet Dev* 1996;**6**(4):432–8.
82. Hogan BLM. Morphogenesis. *Cell* 1999;**96**(2):225–33.
83. Lima de Andrade C., Carvalho MA., Bordin D., da Silva WJ., Del Bel Cury AA., Sotto-Maior BS. Biomechanical Behavior of the Dental Implant Macrodesign. *Int J Oral Maxillofac Implants* 2017;**32**(2):264–70.
84. Martin AD., McCulloch RG. Bone dynamics: stress, strain and fracture. *J Sports Sci* 1987;**5**(2):155–63. Doi: 10.1080/02640418708729773.
85. Schwartz-Dabney CL., Dechow PC. Variations in cortical material properties throughout the human dentate mandible. *Am J Phys Anthropol* 2003;**120**(3):252–77. Doi: 10.1002/ajpa.10121.
86. Katranji A., Misch K., Wang H-L. Cortical Bone Thickness in Dentate and Edentulous Human Cadavers. *J Periodontol* 2007;**78**(5):874–8. Doi: 10.1902/jop.2007.060342.
87. Misch CE., Qu Z., Bidez MW. Mechanical properties of

- trabecular bone in the human mandible: implications for dental implant treatment planning and surgical placement. *J Oral Maxillofac Surg* 1999;**57**(6):700–6; discussion 706–8.
88. Seong W-J., Kim U-K., Swift JQ., Heo Y-C., Hodges JS., Ko C-C. Elastic properties and apparent density of human edentulous maxilla and mandible. *Int J Oral Maxillofac Surg* 2009;**38**(10):1088–93. Doi: 10.1016/j.ijom.2009.06.025.
 89. Peterson J., Wang Q., Dechow PC. Material properties of the dentate maxilla. *Anat Rec Part A Discov Mol Cell Evol Biol* 2006;**288A**(9):962–72. Doi: 10.1002/ar.a.20358.
 90. Parkinson IH., Fazzalari NL. Characterisation of Trabecular Bone Structure. In: Silva MJ, editor. *Skeletal Aging and Osteoporosis*. Springer Berlin Heidelberg; 2013. p. 31–51.
 91. Assunção WG., Gomes EA., Barão VAR., de Sousa EAC. Stress analysis in simulation models with or without implant threads representation. *Int J Oral Maxillofac Implants* 2009.
 92. Frost HM. Skeletal structural adaptations to mechanical usage (SATMU): 1. Redefining Wolff's law: the bone modeling problem. *Anat Rec* 1990;**226**(4):403–13. Doi: 10.1002/ar.1092260402.
 93. Leucht P., Kim J-B., Wazen R., Currey JA., Nanci A., Brunski JB., et al. Effect of mechanical stimuli on skeletal regeneration around implants. *Bone* 2007;**40**(4):919–30. Doi: 10.1016/j.bone.2006.10.027.
 94. Skedros JG., Keenan KE., Williams TJ., Kiser CJ. Secondary osteon size and collagen/lamellar organization (“osteon morphotypes”) are not coupled, but potentially adapt independently for local strain mode or magnitude. *J Struct Biol* 2013. Doi: 10.1016/j.jsb.2012.10.013.
 95. Skedros JG., Mason MW., Nelson MC., Bloebaum RD. Evidence of structural and material adaptation to specific strain features in cortical bone. *Anat Rec* 1996;**246**(1):47–63. Doi: AID-AR6>3.0.CO;2-C.
 96. Zhao E., Xu H., Wang L., Kryczek I., Wu K., Hu Y., et al. Bone marrow and the control of immunity. *Cell Mol Immunol* 2012. Doi: 10.1038/cmi.2011.47.
 97. Karsenty G. Bone as an Endocrine Organ. *Cellular Endocrinology in Health and Disease*. 2014.
 98. Parfitt AM. Bone and plasma calcium homeostasis. *Bone* 1986;**8**:1.
 99. Yelick PC., Zhang W. Mesenchymal stem cells. *Tissue Engineering: Principles and Practices*. 2012.
 100. Rutkovskiy A., Stenslkken K-O., Vaage IJ. Osteoblast Differentiation at a Glance. *Med Sci Monit Basic Res*

- 2016;**22**:95–106. Doi: 10.12659/MSMBR.901142.
101. Franz-Odenaal TA., Hall BK., Witten PE. Buried alive: How osteoblasts become osteocytes. *Dev Dyn* 2006. Doi: 10.1002/dvdy.20603.
 102. Miller SC., Bowman BM., Smith JM., Jee WS. Characterization of endosteal bone-lining cells from fatty marrow bone sites in adult beagles. *Anat Rec* 1980;**198**(2):163–73. Doi: 10.1002/ar.1091980204 [doi].
 103. Stains JP., Civitelli R. Cell-to-cell interactions in bone. *Biochem Biophys Res Commun* 2005;**328**(3):721–7. Doi: 10.1016/j.bbrc.2004.11.078.
 104. Bonewald LF. The amazing osteocyte. *J Bone Miner Res* 2011. Doi: 10.1002/jbmr.320.
 105. Helfrich MH., Ralston SH. *Bone Research Protocols, Methods in Molecular Biology*. vol. 816. Springer Science & Business Media, LLC; 2012.
 106. Bukoreshtliev N V., Haase K., Pelling AE. Mechanical cues in cellular signalling and communication. *Cell Tissue Res* 2013;**352**(1):77–94. Doi: 10.1007/s00441-012-1531-4.
 107. Takano-Yamamoto T. Osteocyte function under compressive mechanical force. *Jpn Dent Sci Rev* 2014;**50**(2):29–39. Doi: 10.1016/j.jdsr.2013.10.004.
 108. Nakashima T., Hayashi M., Fukunaga T., Kurata K., Oh-Hora M., Feng JQ., et al. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat Med* 2011;**17**(10):1231–4. Doi: 10.1038/nm.2452 [doi].
 109. Bonewald LF., Johnson ML. Osteocytes, mechanosensing and Wnt signaling. *Bone* 2008;**42**(4):606–15. Doi: 10.1016/j.bone.2007.12.224 [doi].
 110. Yu K., Sellman DP., Bahraini A., Hagan ML., Elsherbini A., Vanpelt KT., et al. Mechanical loading disrupts osteocyte plasma membranes which initiates mechanosensation events in bone. *J Orthop Res* 2018. Doi: 10.1002/jor.23665.
 111. Boyce BF. Advances in the regulation of osteoclasts and osteoclast functions. *J Dent Res* 2013;**92**(10):860–7. Doi: 10.1177/0022034513500306 [doi].
 112. Takahashi N., Udagawa N., Udagawa N., Suda T. Vitamin D endocrine system and osteoclasts. *Bonekey Rep* 2014;**3**:495. Doi: 10.1038/bonekey.2013.229.
 113. Zhou H., Lu SS., Dempster DW. Bone Remodeling: Cellular Activities in Bone. In: Orwoll ES, Bilezikian JP, and Vanderschueren D, editors. *Osteoporosis in Man. The Effects of Gender on Skeletal Health*. 2nd edition. Elsevier Inc.; 2010. p. 15–24.

114. ADL SM., SIMPSON AGB., FARMER MA., ANDERSEN RA., ANDERSON OR., BARTA JR., et al. The New Higher Level Classification of Eukaryotes with Emphasis on the Taxonomy of Protists. *J Eukaryot Microbiol* 2005;**52**(5):399–451. Doi: 10.1111/j.1550-7408.2005.00053.x.
115. Song RL., Liu XZ., Zhu JQ., Zhang JM., Gao Q., Zhao HY., et al. New roles of filopodia and podosomes in the differentiation and fusion process of osteoclasts. *Genet Mol Res GMR* 2014;**13**(3):4776–87. Doi: 10.4238/2014.July.2.7.
116. Jurdic P., Saltel F., Chabadel A., Destaing O. Podosome and sealing zone: specificity of the osteoclast model. *Eur J Cell Biol* 2006;**85**(3–4):195–202. Doi: 10.1016/j.ejcb.2005.09.008.
117. Luxenburg C., Geblinger D., Klein E., Anderson K., Hanein D., Geiger B., et al. The architecture of the adhesive apparatus of cultured osteoclasts: From podosome formation to sealing zone assembly. *PLoS One* 2007. Doi: 10.1001/jama.1936.02770020060029.
118. Davies JE. Understanding peri-implant endosseous healing. *J Dent Educ* 2003;**67**(8):932–49.
119. Schwartz Z., Boyan BD. Underlying mechanisms at the bone-biomaterial interface. *J Cell Biochem* 1994;**56**(3):340–7. Doi: 10.1002/jcb.240560310.
120. Terheyden H., Lang NP., Bierbaum S., Stadlinger B. Osseointegration--communication of cells. *Clin Oral Implants Res* 2012;**23**(10):1127–35. Doi: 10.1111/j.1600-0501.2011.02327.x.
121. Walsh MC., Takegahara N., Kim H., Choi Y. Updating osteoimmunology: regulation of bone cells by innate and adaptive immunity. *Nat Rev Rheumatol* 2018;**14**(3):146–56. Doi: 10.1038/nrrheum.2017.213.
122. Burns GW., Bottino P. *The Science of Genetics*. 6th ed. New York: Macmillan; 1989.
123. Bosshardt DD., Salvi GE., Huynh-Ba G., Ivanovski S., Donos N., Lang NP. The role of bone debris in early healing adjacent to hydrophilic and hydrophobic implant surfaces in man. *Clin Oral Implants Res* 2011;**22**(4):357–64.
124. Taipale J., Keski-Oja J. Growth factors in the extracellular matrix. *FASEB J* 1997;**11**(1):51–9.
125. Jimbo R., Sawase T., Shibata Y., Hirata K., Hishikawa Y., Tanaka Y., et al. Enhanced osseointegration by the chemotactic activity of plasma fibronectin for cellular fibronectin positive cells. *Biomaterials* 2007;**28**(24):3469–77. Doi: 10.1016/j.biomaterials.2007.04.029.
126. Zhao G., Schwartz Z., Wieland M., Rupp F., Geis-Gerstorfer

- J., Cochran DL., et al. High surface energy enhances cell response to titanium substrate microstructure. *J Biomed Mater Res A* 2005;**74**(1):49–58. Doi: 10.1002/jbm.a.30320 [doi].
127. Wilson CJ., Clegg RE., Leavesley DL., Percy MJ. Mediation of Biomaterial–Cell Interactions by Adsorbed Proteins: A Review. *Tissue Eng* 2005. Doi: 10.1089/ten.2005.11.1.
 128. Furie B., Furie BC. Mechanisms of thrombus formation. *N Engl J Med* 2008;**359**(9):938–49. Doi: 10.1056/NEJMr0801082.
 129. Hoffman M., Monroe DM. Coagulation 2006: a modern view of hemostasis. *Hematol Oncol Clin North Am* 2007;**21**(1):1–11. Doi: 10.1016/j.hoc.2006.11.004.
 130. Gruber R., Karreth F., Kandler B., Fuerst G., Rot A., Fischer MB., et al. Platelet-released supernatants increase migration and proliferation, and decrease osteogenic differentiation of bone marrow-derived mesenchymal progenitor cells under in vitro conditions. *Platelets* 2004. Doi: 10.1080/09537100310001643999.
 131. Agis H., Schröckmair S., Skorianz C., Fischer MB., Watzek G., Gruber R. Platelets increase while serum reduces the differentiation and activity of osteoclasts in vitro. *J Orthop Res* 2013. Doi: 10.1002/jor.22386.
 132. Trindade R., Albrektsson T., Galli S., Prgomet Z., Tengvall P., Wennerberg A. Osseointegration and foreign body reaction: Titanium implants activate the immune system and suppress bone resorption during the first 4 weeks after implantation. *Clin Implant Dent Relat Res* 2018;**20**(1):82–91. Doi: 10.1111/cid.12578.
 133. Neuss S., Schneider RKM., Tietze L., Knüchel R., Jahnhen-Dechent W. Secretion of fibrinolytic enzymes facilitates human mesenchymal stem cell invasion into fibrin clots. *Cells Tissues Organs* 2009. Doi: 10.1159/000215579.
 134. Hotchkiss KM., Sowers KT., Olivares-Navarrete R. Novel in vitro comparative model of osteogenic and inflammatory cell response to dental implants. *Dent Mater Off Publ Acad Dent Mater* 2019;**35**(1):176–84. Doi: 10.1016/j.dental.2018.11.011.
 135. Hotchkiss KM., Reddy GB., Hyzy SL., Schwartz Z., Boyan BD., Olivares-Navarrete R. Titanium surface characteristics, including topography and wettability, alter macrophage activation. *Acta Biomater* 2016;**31**:425–34. Doi: 10.1016/j.actbio.2015.12.003.
 136. Duchene J., Lecomte F., Ahmed S., Cayla C., Pesquero J., Bader M., et al. A novel inflammatory pathway involved in leukocyte recruitment: role for the kinin B1 receptor and the

- chemokine CXCL5. *J Immunol (Baltimore, Md 1950)* 2007;**179**(7):4849–56.
137. Medzhitov R. Origin and physiological roles of inflammation. *Nature* 2008;**454**(7203):428–35. Doi: 10.1038/nature07201.
 138. Aderem A., Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. *Nature* 2000;**406**(6797):782–7. Doi: 10.1038/35021228.
 139. Agrawal S., Agrawal A., Doughty B., Gerwitz A., Blenis J., Van Dyke T., et al. Cutting Edge: Different Toll-Like Receptor Agonists Instruct Dendritic Cells to Induce Distinct Th Responses via Differential Modulation of Extracellular Signal-Regulated Kinase-Mitogen-Activated Protein Kinase and c-Fos. *J Immunol* 2003. Doi: 10.4049/jimmunol.171.10.4984.
 140. Janeway Jr. CA., Travers P., Walport M., Shlomchik MJ. *Immunobiology: the immune system in health and disease*. 6th edition. New York: Garland, cop; 2004.
 141. Mödinger Y., Teixeira GQ., Neidlinger-Wilke C., Ignatius A. Role of the Complement System in the Response to Orthopedic Biomaterials. *Int J Mol Sci* 2018;**19**(11):3367. Doi: 10.3390/ijms19113367.
 142. Cesarman-Maus G., Hajjar KA. Molecular mechanisms of fibrinolysis. *Br J Haematol* 2005. Doi: 10.1111/j.1365-2141.2005.05444.x.
 143. De Witt SM., Verdoold R., Cosemans JMEM., Heemskerck JWM. Insights into platelet-based control of coagulation. *Thromb Res* 2014. Doi: 10.1016/S0049-3848(14)50024-2.
 144. Wynn TA., Chawla A., Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature* 2013. Doi: 10.1038/nature12034.
 145. Mantovani A., Sica A., Sozzani S., Allavena P., Vecchi A., Locati M. The chemokine system in diverse forms of macrophage activation and polarization 2004;**25**(12):677–86.
 146. Martinez FO., Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep* 2014;**6**:13.
 147. Chistiakov DA., Myasoedova VA., Revin V V., Orekhov AN., Bobryshev Y V. The impact of interferon-regulatory factors to macrophage differentiation and polarization into M1 and M2. *Immunobiology* 2017. Doi: 10.1016/j.imbio.2017.10.005.
 148. Medzhitov R., Janeway Jr CA. Innate immunity: impact on the adaptive immune response. *Curr Opin Immunol* 1998;**9**(1):4–9. Doi: S0952-7915(97)80152-5 [pii].
 149. Nomura T., Ishii A., Shimizu H., Taguchi N., Yoshie H., Kusakari H., et al. Tissue inhibitor of metalloproteinases-1,

- matrix metalloproteinases-1 and -8, and collagenase activity levels in peri-implant crevicular fluid after implantation. *Clin Oral Implants Res* 2000;**11**(5):430–40. Doi: clr110505 [pii].
150. Alexander KA., Chang MK., Maylin ER., Kohler T., Müller R., Wu AC., et al. Osteal macrophages promote in vivo intramembranous bone healing in a mouse tibial injury model. *J Bone Miner Res Off J Am Soc Bone Miner Res* 2011;**26**(7):1517–32. Doi: 10.1002/jbmr.354.
 151. Miron RJ., Bosshardt DD. OsteoMacs: Key players around bone biomaterials. *Biomaterials* 2016. Doi: 10.1016/j.biomaterials.2015.12.017.
 152. Berglundh., Abrahamsson I., Lang NP., Lindhe J. De novo alveolar bone formation adjacent to endosseous implants: A model study in the dog. *Clin Oral Implants Res* 2003;**14**(3):251–62. Doi: 10.1034/j.1600-0501.2003.00972.x.
 153. Hitchon C., Wong K., Ma G., Reed J., Lyttle D., El-Gabalawy H. Hypoxia-induced production of stromal cell-derived factor 1 (CXCL12) and vascular endothelial growth factor by synovial fibroblasts. *Arthritis Rheum* 2002. Doi: 10.1002/art.10520.
 154. Wierzbicka-Patynowski I., Schwarzbauer JE. The ins and outs of fibronectin matrix assembly. *J Cell Sci* 2003;**116**(Pt 16):3269–76. Doi: 10.1242/jcs.00670 [doi].
 155. Albrektsson T., Wennerberg A., Hannig M., Hannig C., Bougas K., Rasouli R., et al. Bidirectional Notch Signaling and Osteocyte-Derived Factors in the Bone Marrow Microenvironment Promote Tumor Cell Proliferation and Bone Destruction in Multiple Myeloma. *Clin Oral Implants Res* 2018;**9**(2):262–6. Doi: 10.1111/clr.13375.
 156. Huntley R., Jensen E., Gopalakrishnan R., Mansky KC. Bone morphogenetic proteins: Their role in regulating osteoclast differentiation. *Bone Reports* 2019. Doi: 10.1016/j.bonr.2019.100207.
 157. Shui C., Spelsberg TC., Riggs BL., Khosla S. Changes in Runx2/Cbfa1 Expression and Activity During Osteoblastic Differentiation of Human Bone Marrow Stromal Cells. *J Bone Miner Res* 2003;**18**(2):213–21. Doi: 10.1359/jbmr.2003.18.2.213.
 158. Ohata Y., Ozono K. [Bone and Stem Cells. The mechanism of osteogenic differentiation from mesenchymal stem cell]. *Clin Calcium* 2014;**24**(4):501–8. Doi: CliCa1404501508.
 159. Strauss FJ., Di Summa F., Stähli A., Matos L., Vaca F., Schuldt G., et al. TGF- β activity in acid bone lysate adsorbs to titanium surface. *Clin Implant Dent Relat Res* 2019. Doi:

- 10.1111/cid.12734.
160. Nusse R. Wnt signaling in disease and in development. *Cell Res* 2005;**15**(1):28–32. Doi: 10.1038/sj.cr.7290260.
 161. Kobayashi Y., Uehara S., Udagawa N., Takahashi N. Regulation of bone metabolism by Wnt signals. *J Biochem* 2016;**159**(4):387–92. Doi: 10.1093/jb/mvv124.
 162. Itasaki N., Hoppler S. Crosstalk between Wnt and bone morphogenic protein signaling: a turbulent relationship. *Dev Dyn An Off Publ Am Assoc Anat* 2010;**239**(1):16–33. Doi: 10.1002/dvdy.22009.
 163. Yang G., Fang W., Liu T., He F., Chen X., Zhou Y., et al. Gene expression profiling of bone marrow-derived stromal cells seeded onto a sandblasted, large-grit, acid-etched-treated titanium implant surface: The role of the Wnt pathway. *Arch Oral Biol* 2016;**61**:71–8.
 164. Olivares-Navarrete R., Hyzy S., Wieland M., Boyan BD., Schwartz Z. The roles of Wnt signaling modulators Dickkopf-1 (Dkk1) and Dickkopf-2 (Dkk2) and cell maturation state in osteogenesis on microstructured titanium surfaces. *Biomaterials* 2010;**31**(8):2015–24. Doi: 10.1016/j.biomaterials.2009.11.071.
 165. Olivares-Navarrete R., Hyzy SL., Hutton DL., Dunn GR., Appert C., Boyan BD., et al. Role of non-canonical Wnt signaling in osteoblast maturation on microstructured titanium surfaces. *Acta Biomater* 2011;**7**(6):2740–50. Doi: 10.1016/j.actbio.2011.02.030.
 166. Boyan BD., Olivares-Navarrete R., Berger MB., Hyzy SL., Schwartz Z. Role of Wnt11 during Osteogenic Differentiation of Human Mesenchymal Stem Cells on Microstructured Titanium Surfaces. *Sci Rep* 2018;**8**(1):8588. Doi: 10.1038/s41598-018-26901-8.
 167. Sims NA., Gooi JH. Bone remodeling: Multiple cellular interactions required for coupling of bone formation and resorption. *Seminars in cell & developmental biology*, vol. 19. Elsevier; 2008. p. 444–51.
 168. Cölfen H. Biomineralization: A crystal-clear view. *Nat Mater* 2010;**9**(12):960–1. Doi: 10.1038/nmat2911.
 169. Eriksen EF. Cellular mechanisms of bone remodeling. *Rev Endocr Metab Disord* 2010;**11**(4):219–27. Doi: 10.1007/s11154-010-9153-1.
 170. Duyck J., Corpas L., Vermeiren S., Ogawa T., Quirynen M., Vandamme K., et al. Histological, histomorphometrical, and radiological evaluation of an experimental implant design with a high insertion torque. *Clin Oral Implants Res*

- 2010;**21**(8):877–84.
171. Abrahamsson I., Berglundh T., Linder E., Lang NP., Lindhe J. Early bone formation adjacent to rough and turned endosseous implant surfaces. An experimental study in the dog. *Clin Oral Implants Res* 2004;**15**(4):381–92. Doi: 10.1111/j.1600-0501.2004.01082.x [doi].
 172. Gomes JB., Campos FE., Marin C., Teixeira HS., Bonfante EA., Suzuki M., et al. Implant biomechanical stability variation at early implantation times in vivo: an experimental study in dogs. *Int J Oral Maxillofac Implants* 2013;**28**(3):128. Doi: 10.11607/jomi.2885 [doi].
 173. Raghavendra S., Wood MC., Taylor TD. Early wound healing around endosseous implants: a review of the literature. *Int J Oral Maxillofac Implants* 2005;**20**(3):425–31.
 174. Baltayan S., Pi-Anfruns J., Aghaloo T., Moy PK. The predictive value of resonance frequency analysis measurements in the surgical placement and loading of endosseous implants. *J Oral Maxillofac Surg* 2016. Doi: 10.1016/j.joms.2016.01.048.
 175. Suzuki S., Kobayashi H., Ogawa T. Implant stability change and osseointegration speed of immediately loaded photofunctionalized implants. *Implant Dent* 2013. Doi: 10.1097/ID.0b013e31829deb62.
 176. Stocchero., Toia M., Cecchinato D., Becktor JP., Coelho PG., Jimbo R. Biomechanical, biologic, and clinical outcomes of undersized implant surgical preparation: A systematic review. *Int J Oral Maxillofac Implant* 2016;**31**(6):1247–63. Doi: 10.11607/jomi.5340.
 177. Boyle WJ., Simonet WS., Lacey DL. Osteoclast differentiation and activation. *Nature* 2003;**423**(6937):337–42. Doi: 10.1038/nature01658 [doi].
 178. Kwon OH., Lee C-K., Lee YI., Paik S-G., Lee H-J. The hematopoietic transcription factor PU.1 regulates RANK gene expression in myeloid progenitors. *Biochem Biophys Res Commun* 2005;**335**(2):437–46. Doi: 10.1016/j.bbrc.2005.07.092.
 179. Umeda S., Takahashi K., Shultz LD., Naito M., Takagi K. Effects of macrophage colony-stimulating factor on macrophages and their related cell populations in the osteopetrosis mouse defective in production of functional macrophage colony-stimulating factor protein. *Am J Pathol* 1996;**149**(2):559–74.
 180. Roberts WE., Roberts JA., Epker BN., Burr DB., Hartsfield Jr JK. Remodeling of mineralized tissues, part I: the frost legacy
. *Semin Orthod* 2006;**12**:216–37.

181. Simonet WS., Lacey DL., Dunstan CR., Kelley M., Chang MS., Lthy R., et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997;**89**(2):309–19.
182. Kang JH., Ko HM., Moon JS., Yoo HI., Jung JY., Kim MS., et al. Osteoprotegerin expressed by osteoclasts: An autoregulator of osteoclastogenesis. *J Dent Res* 2014. Doi: 10.1177/0022034514552677.
183. Li J., Sarosi I., Yan X-Q., Morony S., Capparelli C., Tan H-L., et al. RANK Is the Intrinsic Hematopoietic Cell Surface Receptor That Controls Osteoclastogenesis and Regulation of Bone Mass and Calcium Metabolism. *Proc Natl Acad Sci U S A* 2000;**97**(4):1566–71. Doi: 10.1073/pnas.97.4.1566.
184. Takahashi N., Udagawa N., Suda T. A new member of tumor necrosis factor ligand family, ODF/OPGL/TRANCE/RANKL, regulates osteoclast differentiation and function. *Biochem Biophys Res Commun* 1999;**256**(3):449–55. Doi: 10.1006/bbrc.1999.0252.
185. Anderson DM., Maraskovsky E., Billingsley WL., Dougall WC., Tometsko ME., Roux ER., et al. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* 1997;**390**(6656):175–9. Doi: 10.1038/36593.
186. Udagawa N., Takahashi N., Jimi E., Matsuzaki K., Tsurukai T., Itoh K., et al. Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. *Bone* 1999;**25**(5):517–23. Doi: S8756328299002100 [pii].
187. Xiong J., Onal M., Jilka RL., Weinstein RS., Manolagas SC., O'Brien CA. Matrix-embedded cells control osteoclast formation. *Nat Med* 2011;**17**(10):1235–41. Doi: 10.1038/nm.2448 [doi].
188. Wong BR., Josien R., Lee SY., Vologodskaia M., Steinman RM., Choi Y. The TRAF family of signal transducers mediates NF-kappaB activation by the TRANCE receptor. *J Biol Chem* 1998;**273**(43):28355–9.
189. Boyce BF., Xiu Y., Li J., Xing L., Yao Z. NF-B-Mediated Regulation of Osteoclastogenesis. *Endocrinol Metab (Seoul, Korea)* 2015;**30**(1):35–44. Doi: 10.3803/EnM.2015.30.1.35.
190. Boyce BF., Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Arch Biochem Biophys* 2008;**473**(2):139–46. Doi: 10.1016/j.abb.2008.03.018.
191. Parfitt AM. Targeted and nontargeted bone remodeling:

- relationship to basic multicellular unit origination and progression. *Bone* 2002;**30**(1):5–7.
192. Riihonen R., Supuran CT., Parkkila S., Pastorekova S., Väänänen HK., Laitala-Leinonen T. Membrane-bound carbonic anhydrases in osteoclasts. *Bone* 2007. Doi: 10.1016/j.bone.2006.11.028.
 193. Halleen JM., Alatalo SL., Suominen H., Cheng S., Janckila AJ., Väänänen HK. Tartrate-resistant acid phosphatase 5b: A novel serum marker of bone resorption. *J Bone Miner Res* 2000. Doi: 10.1359/jbmr.2000.15.7.1337.
 194. Hayman AR., Cox TM. Purple acid phosphatase of the human macrophage and osteoclast. Characterization, molecular properties, and crystallization of the recombinant di-iron-oxo protein secreted by baculovirus-infected insect cells. *J Biol Chem* 1994;**269**(2):1294–300.
 195. Vääräniemi J., Halleen JM., Kaarlonen K., Ylipahkala H., Alatalo SL., Andersson G., et al. Intracellular machinery for matrix degradation in bone-resorbing osteoclasts. *J Bone Miner Res* 2004. Doi: 10.1359/JBMR.040603.
 196. Troen BR. The regulation of cathepsin K gene expression. *Annals of the New York Academy of Sciences*. 2006.
 197. Salo J., Lehenkari P., Mulari M., Metsikkö K., Väänänen HK. Removal of osteoclast bone resorption products by transcytosis. *Science* (80-) 1997. Doi: 10.1126/science.276.5310.270.
 198. Sakai S., Takaishi H., Matsuzaki K., Kaneko H., Furukawa M., Miyauchi Y., et al. 1-Alpha, 25-dihydroxy vitamin D3 inhibits osteoclastogenesis through IFN-beta-dependent NFATc1 suppression. *J Bone Miner Metab* 2009;**27**(6):643–52. Doi: 10.1007/s00774-009-0084-4 [doi].
 199. Kogawa M., Findlay DM., Anderson PH., Ormsby R., Vincent C., Morris HA., et al. Osteoclastic metabolism of 25(OH)-vitamin D3: a potential mechanism for optimization of bone resorption. *Endocrinology* 2010;**151**(10):4613–25. Doi: 10.1210/en.2010-0334 [doi].
 200. Fu Q., Jilka RL., Manolagas SC., O'Brien CA. Parathyroid hormone stimulates receptor activator of NFkappa B ligand and inhibits osteoprotegerin expression via protein kinase A activation of cAMP-response element-binding protein. *J Biol Chem* 2002;**277**(50):48868–75. Doi: 10.1074/jbc.M208494200 [doi].
 201. Zhou C., Li Y., Wang X., Shui X., Hu J. 1,25Dihydroxy vitamin D(3) improves titanium implant osseointegration in osteoporotic rats. *Oral Surg Oral Med Oral Pathol Oral Radiol*

- 2012;**114**(5 Suppl):174. Doi: 10.1016/j.oooo.2011.09.030 [doi].
202. Zaidi M., Moonga BS., Abe E. Calcitonin and bone formation: a knockout full of surprises. *J Clin Invest* 2002;**110**(12):1769–71. Doi: 10.1172/JCI17425.
203. Keller J., Catala-Lehnen P., Huebner AK., Jeschke A., Heckt T., Lueth A., et al. Calcitonin controls bone formation by inhibiting the release of sphingosine 1-phosphate from osteoclasts. *Nat Commun* 2014. Doi: 10.1038/ncomms6215.
204. Yeon J-T., Choi S-W., Kim SH. Arginase 1 is a negative regulator of osteoclast differentiation. *Amino Acids* 2016;**48**(2):559–65. Doi: 10.1007/s00726-015-2112-0.
205. Lassen NE., Andersen TL., Pløen GG., Søre K., Hauge EM., Harving S., et al. Coupling of Bone Resorption and Formation in Real Time: New Knowledge Gained From Human Haversian BMUs. *J Bone Miner Res* 2017;**32**(7):1395–405. Doi: 10.1002/jbmr.3091.
206. Murshed M., McKee MD. Molecular determinants of extracellular matrix mineralization in bone and blood vessels. *Curr Opin Nephrol Hypertens* 2010;**19**(4):359–65. Doi: 10.1097/MNH.0b013e3283393a2b.
207. Weiner S., Traub W. Organization of hydroxyapatite crystals within collagen fibrils. *FEBS Lett* 1986;**206**(2):262–6.
208. Palma-Carrió C., Maestre-Ferrín L., Peñarrocha-Oltra D., Peñarrocha-Diogo MA., Peñarrocha-Diogo M. Risk factors associated with early failure of dental implants. A literature review. *Med Oral Patol Oral Cir Bucal* 2011. Doi: 10.4317/medoral.16.e514.
209. Krennmair G., Seemann R., Piehslinger E. Dental implants in patients with rheumatoid arthritis: Clinical outcome and peri-implant findings. *J Clin Periodontol* 2010. Doi: 10.1111/j.1600-051X.2010.01606.x.
210. Javed F., Romanos GE. Impact of diabetes mellitus and glycemic control on the osseointegration of dental implants: a systematic literature review. *J Periodontol* 2009;**80**(11):1719–30. Doi: 10.1902/jop.2009.090283 [doi].
211. Naujokat H., Kunzendorf B., Wiltfang J. Dental implants and diabetes mellitus—a systematic review. *Int J Implant Dent* 2016. Doi: 10.1186/s40729-016-0038-2.
212. Chrcanovic BR., Albrektsson T., Wennerberg A. Dental implants in irradiated versus nonirradiated patients: A meta-analysis. *Head Neck* 2016. Doi: 10.1002/hed.23875.
213. Javed F., Romanos GE. Dental Implants in Patients with Crohn's Disease. In: Javed F, and Romanos GE, editors.

Evidence-based Implant Dentistry and Systemic Conditions. Hoboken, USA: Wiley-Blackwell; 2018. p. 13.

214. Chrcanovic BR., Kisch J., Albrektsson T., Wennerberg A. Factors Influencing Early Dental Implant Failures. *J Dent Res* 2016;**95**(9):995–1002. Doi: 10.1177/0022034516646098.
215. Chrcanovic B., Kisch J., Albrektsson T., Wennerberg A. Intake of Proton Pump Inhibitors Is Associated with an Increased Risk of Dental Implant Failure. *Int J Oral Maxillofac Implants* 2017. Doi: 10.11607/jomi.5662.
216. Yajima N., Munakata M., Fuchigami K., Sanda M., Kasugai S. Influence of Bisphosphonates on Implant Failure Rates and Characteristics of Postmenopausal Woman Mandibular Jawbone. *J Oral Implantol* 2017. Doi: 10.1563/aaid-joi-D-17-00015.
217. Chrcanovic BR., Albrektsson T., Wennerberg A. Reasons for failures of oral implants. *J Oral Rehabil* 2014;**41**(6):443–76. Doi: 10.1111/joor.12157.
218. Chrcanovic B., Kisch J., Albrektsson T., Wennerberg A. Impact of Different Surgeons on Dental Implant Failure. *Int J Prosthodont* 2017. Doi: 10.11607/ijp.5151.
219. Renvert S., Persson GR., Pirih FQ., Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. *J Clin Periodontol* 2018. Doi: 10.1111/jcpe.12956.
220. Chrcanovic BR., Albrektsson T., Wennerberg A. Smoking and dental implants: A systematic review and meta-analysis. *J Dent* 2015. Doi: 10.1016/j.jdent.2015.03.003.
221. Chrcanovic BR., Kisch J., Albrektsson T., Wennerberg A. Bruxism and dental implant treatment complications: a retrospective comparative study of 98 bruxer patients and a matched group. *Clin Oral Implants Res* 2017. Doi: 10.1111/clr.12844.
222. Sakka S., Baroudi K., Nassani MZ. Factors associated with early and late failure of dental implants. *J Investig Clin Dent* 2012. Doi: 10.1111/j.2041-1626.2012.00162.x.
223. Chrcanovic BR., Kisch J., Albrektsson T., Wennerberg A. Analysis of risk factors for cluster behavior of dental implant failures. *Clin Implant Dent Relat Res* 2017;**19**(4):632–42. Doi: 10.1111/cid.12485.
224. Carranza FA. Bone Loss and Patterns of Bone Destruction. In: Newman MG, Takei HH, and Carranza FA, editors. *Carranza's Clinical Periodontology*. 9th ed. Philadelphia, USA: W B Saunders Co-Elsevier Inc; 2002. p. 363.
225. Araújo MG., Lindhe J. Dimensional ridge alterations following

- tooth extraction. An experimental study in the dog. *J Clin Periodontol* 2005. Doi: 10.1111/j.1600-051X.2005.00642.x.
226. Schropp L., Wenzel A., Kostopoulos L., Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. *Int J Periodontics Restorative Dent* 2003;**23**(4):313–23.
 227. Avila-Ortiz G., Elangovan S., Kramer KWO., Blanchette D., Dawson D V. Effect of alveolar ridge preservation after tooth extraction: A systematic review and meta-analysis. *J Dent Res* 2014. Doi: 10.1177/0022034514541127.
 228. Tan WL., Wong TLT., Wong MCM., Lang NP. A systematic review of post-extraction alveolar hard and soft tissue dimensional changes in humans. *Clin Oral Implants Res* 2012;**23 Suppl 5**:1–21. Doi: 10.1111/j.1600-0501.2011.02375.x.
 229. Bertl K., Kukla EB., Albugami R., Beck F., Gahleitner A., Stavropoulos A. Timeframe of socket cortication after tooth extraction: A retrospective radiographic study. *Clin Oral Implants Res* 2018;**29**(1):130–8. Doi: 10.1111/clr.13081.
 230. Bartold PM., Cantley MD., Haynes DR. Mechanisms and control of pathologic bone loss in periodontitis. *Periodontol* 2000 2010;**53**(1):55–69. Doi: 10.1111/j.1600-0757.2010.00347.x.
 231. Lang NP. *Proceedings of the 1st European Workshop on Periodontology*. Quintessence Publishing Company; 1994.
 232. Schnitman PA., Shulman LB. Recommendations of the consensus development conference on dental implants. *J Am Dent Assoc* 1979;**98**(3):373–7.
 233. Albrektsson T., Zarb G., Worthington P., Eriksson AR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. *Int J Oral Maxillofac Implants* 1986;**1**(1):11–25.
 234. Albrektsson T., Zarb GA. Determinants of correct clinical reporting. *Int J Prosthodont* 1998;**11**(5):517–21.
 235. Buser D., Weber HP., Lang NP. Tissue integration of non-submerged implants. 1-year results of a prospective study with 100 ITI hollow-cylinder and hollow-screw implants. *Clin Oral Implants Res* 1990;**1**(1):33–40.
 236. Misch CE., Perel ML., Wang HL., Sammartino G., Galindo-Moreno P., Trisi P., et al. Implant success, survival, and failure: The International Congress of Oral Implantologists (ICOI) pisa consensus conference. *Implant Dent* 2008.
 237. Albrektsson T., Zarb GA. Current Interpretations of the Osseointegrated Response: Clinical Significance. *Int J*

- Prosthodont* 1993. Doi: 10.1023/A:1010135400465.
238. Araujo MG., Lindhe J. Peri-implant health. *J Clin Periodontol* 2018;**45**:S230–6. Doi: 10.1111/jcpe.12952.
 239. Molina A., Sanz-Sánchez I., Martín C., Blanco J., Sanz M. The effect of one-time abutment placement on interproximal bone levels and peri-implant soft tissues: a prospective randomized clinical trial. *Clin Oral Implants Res* 2017. Doi: 10.1111/clr.12818.
 240. Berglundh T., Armitage G., Araujo MG., Avila-Ortiz G., Blanco J., Camargo PM., et al. *Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions*. vol. 45. 2018.
 241. Chiapasco M., Casentini P., Zaniboni DDSM. Bone Augmentation Procedures in Implant Dentistry. *Int J Oral Maxillofac Implants* 2009. Doi: 10.1016/0029-7844(94)00457-O.
 242. Retzepi M., Donos N. Guided Bone Regeneration: Biological principle and therapeutic applications. *Clin Oral Implants Res* 2010. Doi: 10.1111/j.1600-0501.2010.01922.x.
 243. Buser D., Chappuis V., Kuchler U., Bornstein MM., Wittneben JG., Buser R., et al. Long-term stability of early implant placement with contour augmentation. *J Dent Res* 2013;**92**(12 Suppl):82S. Doi: 10.1177/0022034513504949 [doi].
 244. Chen F-M., Zhang J., Zhang M., An Y., Chen F., Wu Z-F. A review on endogenous regenerative technology in periodontal regenerative medicine. *Biomaterials* 2010;**31**(31):7892–927.
 245. Miron RJ., Zhang YF. Osteoinduction: A review of old concepts with new standards. *J Dent Res* 2012. Doi: 10.1177/0022034511435260.
 246. Lopez CD., Diaz-Siso JR., Witek L., Bekisz JM., Cronstein BN., Torroni A., et al. Three dimensionally printed bioactive ceramic scaffold osseointegration across critical-sized mandibular defects. *J Surg Res* 2018. Doi: 10.1016/j.jss.2017.10.027.
 247. Wennerberg A., Jimbo R., Stübinger S., Obrecht M., Dard M., Berner S. Nanostructures and hydrophilicity influence osseointegration: a biomechanical study in the rabbit tibia. *Clin Oral Implants Res* 2014;**25**(9):1041–50. Doi: 10.1111/clr.12213.
 248. Rupp F., Gittens RA., Scheideler L., Marmur A., Boyan BD., Schwartz Z., et al. A review on the wettability of dental implant surfaces I: theoretical and experimental aspects. *Acta Biomater* 2014;**10**(7):2894–906. Doi:

- 10.1016/j.actbio.2014.02.040.
249. Gittens RA., Scheideler L., Rupp F., Hyzy SL., Geis-Gerstorfer J., Schwartz Z., et al. A review on the wettability of dental implant surfaces II: Biological and clinical aspects. *Acta Biomater* 2014;**10**(7):2907–18. Doi: 10.1016/j.actbio.2014.03.032.
 250. Mendonça G., Mendonça DBS., Aragao FJL., Cooper LF. Advancing dental implant surface technology—from micron-to nanotopography. *Biomaterials* 2008;**29**(28):3822–35.
 251. Dohan Ehrenfest DM., Coelho PG., Kang B-S., Sul Y-T., Albrektsson T. Classification of osseointegrated implant surfaces: materials, chemistry and topography. *Trends Biotechnol* 2010;**28**(4):198–206. Doi: 10.1016/j.tibtech.2009.12.003.
 252. Wennerberg A., Albrektsson T., Jimbo R. Overview of Surface Microtopography/Chemistry/Physics/Nano-roughness. In: Wennerberg A, Albrektsson T, and Jimbo R, editors. *Implant Surfaces and Their Biological and Clinical Impact*. Springer; 2015. p. 7.
 253. Kokubo T., Kim HM., Kawashita M., Nakamura T. Bioactive metals: preparation and properties. *J Mater Sci Mater Med* 2004;**15**(2):99–107.
 254. Zhao G., Raines AL., Wieland M., Schwartz Z., Boyan BD. Requirement for both micron-and submicron scale structure for synergistic responses of osteoblasts to substrate surface energy and topography. *Biomaterials* 2007;**28**(18):2821–9.
 255. Anselme K., Biggerelle M. Topography effects of pure titanium substrates on human osteoblast long-term adhesion. *Acta Biomater* 2005;**1**(2):211–22. Doi: S1742-7061(04)00033-9 [pii].
 256. Wennerberg A. The importance of surface roughness for implant incorporation 1998;**38**(5–6):657–62.
 257. Boyan BD., Cheng A., Olivares-Navarrete R., Schwartz Z. Implant Surface Design Regulates Mesenchymal Stem Cell Differentiation and Maturation. *Adv Dent Res* 2016. Doi: 10.1177/0022034515624444.
 258. Baron R., Neff L., Louvard D., Courtoy PJ. Cell-mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of a 100-kD lysosomal membrane protein at the osteoclast ruffled border. *J Cell Biol* 1985;**101**(6):2210–22.
 259. Chambers TJ., Revell PA., Fuller K., Athanasou NA. Resorption of bone by isolated rabbit osteoclasts. *J Cell Sci* 1984;**66**:383–99.

260. Boyan BD., Schwartz Z., Lohmann CH., Sylvia VL., Cochran DL., Dean DD., et al. Pretreatment of bone with osteoclasts affects phenotypic expression of osteoblast-like cells. *J Orthop Res Off Publ Orthop Res Soc* 2003;**21**(4):638–47. Doi: 10.1016/S0736-0266(02)00261-9.
261. Wennerberg A., Albrektsson T., Jimbo R. Overview of Surface Evaluation Techniques. In: Wennerberg A, Albrektsson T, and Jimbo R, editors. *Implant Surfaces and Their Biological and Clinical Impact*. Springer; 2015. p. 1–5.
262. Wennerberg A., Albrektsson T. Suggested guidelines for the topographic evaluation of implant surfaces. *Int J Oral Maxillofac Implants* 2000;**15**(3).
263. Vercaigne S., Wolke JG., Naert I., Jansen JA. Histomorphometrical and mechanical evaluation of titanium plasma-spray-coated implants placed in the cortical bone of goats. *J Biomed Mater Res* 1998;**41**(1):41–8. Doi: 10.1002/(SICI)1097-4636(199807)41:1<41::AID-JBM5>3.0.CO;2-Q [pii].
264. Astrand P., Engquist B., Anzén B., Bergendal T., Hallman M., Karlsson U., et al. A three-year follow-up report of a comparative study of ITI Dental Implants and Brånemark System implants in the treatment of the partially edentulous maxilla. *Clin Implant Dent Relat Res* 2004;**6**(3):130–41.
265. Rocuzzo M., Bunino M., Prioglio F., Bianchi SD. Early loading of sandblasted and acid-etched (SLA) implants: a prospective split-mouth comparative study: One-year results. *Clin Oral Implants Res* 2001;**12**(6):572–8. Doi: 10.1034/j.1600-0501.2001.120604.x.
266. Buser D., Nydegger T., Oxland T., Cochran DL., Schenk RK., Hirt HP., et al. Interface shear strength of titanium implants with a sandblasted and acid-etched surface: a biomechanical study in the maxilla of miniature pigs. *J Biomed Mater Res* 1999;**45**(2):75–83. Doi: AID-JBM1>3.0.CO;2-P [pii].
267. Al-Nawas B., Hangen U., Duschner H., Krummenauer F., Wagner W. Turned, machined versus double-etched dental implants in vivo. *Clin Implant Dent Relat Res* 2007;**9**(2):71–8. Doi: 10.1111/j.1708-8208.2007.00030.x.
268. Nygren H., Eriksson C., Lausmaa J. Adhesion and activation of platelets and polymorphonuclear granulocyte cells at TiO₂ surfaces. *J Lab Clin Med* 1997. Doi: 10.1016/S0022-2143(97)90159-1.
269. Wieland M. Experimental determination and quantitative evaluation of the surface composition and topography of medical implant surfaces and their influence on osteoblastic

- cell-surface interactions. Doi: 10.3929/ethz-a-003845739.
270. Andrukhov O., Huber R., Shi B., Berner S., Rausch-Fan X., Moritz A., et al. Proliferation, behavior, and differentiation of osteoblasts on surfaces of different microroughness. *Dent Mater Off Publ Acad Dent Mater* 2016;**32**(11):1374–84. Doi: 10.1016/j.dental.2016.08.217.
 271. Buser D., Schenk RK., Steinemann S., Fiorellini JP., Fox CH., Stich H. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. *J Biomed Mater Res* 1991;**25**(7):889–902. Doi: 10.1002/jbm.820250708 [doi].
 272. Coelho PG., Bonfante EA., Pessoa RS., Marin C., Granato R., Giro G., et al. Characterization of Five Different Implant Surfaces and Their Effect on Osseointegration: A Study in Dogs. *J Periodontol* 2011;**82**(5):742–50. Doi: 10.1902/jop.2010.100520.
 273. Dunn GA., Ebendal T. Contact guidance on oriented collagen gels. *Exp Cell Res* 1978;**111**(2):475–9.
 274. Gittens RA., McLachlan T., Olivares-Navarrete R., Cai Y., Berner S., Tannenbaum R., et al. The effects of combined micron-/submicron-scale surface roughness and nanoscale features on cell proliferation and differentiation. *Biomaterials* 2011;**32**(13):3395–403. Doi: 10.1016/j.biomaterials.2011.01.029 [doi].
 275. Hotchkiss KM., Ayad NB., Hyzy SL., Boyan BD., Olivares-Navarrete R. Dental implant surface chemistry and energy alter macrophage activation in vitro. *Clin Oral Implants Res* 2017;**28**(4):414–23. Doi: 10.1111/clr.12814.
 276. Lee C-H., Kim Y-J., Jang J-H., Park J-W. Modulating macrophage polarization with divalent cations in nanostructured titanium implant surfaces. *Nanotechnology* 2016;**27**(8):085101. Doi: 10.1088/0957-4484/27/8/085101.
 277. Mendonça G., Mendonça D., Aragao FJL., Cooper LF. The combination of micron and nanotopography by H2SO4/H2O2 treatment and its effects on osteoblast-specific gene expression of hMSCs. *J Biomed Mater Res Part A* 2010;**94**(1):169–79.
 278. Annunziata M., Oliva A., Buosciolo A., Giordano M., Guida A., Guida L. Bone marrow mesenchymal stem cell response to nano-structured oxidized and turned titanium surfaces. *Clin Oral Implants Res* 2012;**23**(6):733–40. Doi: 10.1111/j.1600-0501.2011.02194.x.
 279. Gittens RA., Olivares-Navarrete R., McLachlan T., Cai Y., Hyzy SL., Schneider JM., et al. Differential responses of osteoblast lineage cells to nanotopographically-modified,

- microroughened titanium-aluminum-vanadium alloy surfaces. *Biomaterials* 2012;**33**(35):8986–94.
280. Hannig M., Hannig C. Nanomaterials in preventive dentistry. *Nat Nanotechnol* 2010;**5**(8):565–9. Doi: 10.1038/nnano.2010.83.
 281. Lang NP., Matinlinna JP. Titanium in Implant Dentistry. In: Matinlinna JP, editor. *Handbook of Oral Biomaterials*. Singapore: Pan Stanford Publishing; 2014. p. 339–58.
 282. Textor M., Sittig C., Frauchiger V., Tosatti S., Brunette DM. Properties and Biological Significance of Natural Oxide Films on Titanium and Its Alloys. In: Brunette DM, Tengvall P, Textor M, and Thomson P, editors. *Titanium in Medicine*. Berlin: Springer-Verlag; 2001. p. 171–230.
 283. Aronsson BO., Lausmaa J., Kasemo B. Glow discharge plasma treatment for surface cleaning and modification of metallic biomaterials. *J Biomed Mater Res* 1997;**35**(1):49–73.
 284. DePalma VA., Baier RE., Ford JW., Glott VL., Furuse A. Investigation of three-surface properties of several metals and their relation to blood compatibility. *J Biomed Mater Res* 1972;**6**(4):37–75. Doi: 10.1002/jbm.820060406.
 285. Rupp F., Scheideler L., Olshanska N., de Wild M., Wieland M., Geis-Gerstorfer J. Enhancing surface free energy and hydrophilicity through chemical modification of microstructured titanium implant surfaces. *J Biomed Mater Res A* 2006;**76**(2):323–34. Doi: 10.1002/jbm.a.30518.
 286. Lung CYK., Matinlinna JP., Kukk E., Hägerth T. Surface modifications of zirconia by various chemical treatments. *Appl Surf Sci* 2010;**257**:1228–35.
 287. Baier RE., Meyer AE. Implant surface preparation. *Int J Oral Maxillofac Implants* 1988;**3**(1):9–20.
 288. McBain JW., Porter JL., Sessions RF. The Nature of the Sorption of Water by Charcoal. *J Am Chem Soc* 1933;**55**(6):2294–304. Doi: 10.1021/ja01333a014.
 289. Marković Z., Lustig A., Engel J., Richter H., Hörmann H. Shape and stability of fibronectin in solutions of different pH and ionic strength. *Hoppe Seylers Z Physiol Chem* 1983;**364**(12):1795–804.
 290. Bergkvist M., Carlsson J., Oscarsson S. Surface-dependent conformations of human plasma fibronectin adsorbed to silica, mica, and hydrophobic surfaces, studied with use of Atomic Force Microscopy. *J Biomed Mater Res A* 2003;**64**(2):349–56. Doi: 10.1002/jbm.a.10423.
 291. Baier RE., Meyer AE., Natiella JR., Natiella RR., Carter JM. Surface properties determine bioadhesive outcomes: methods

- and results. *J Biomed Mater Res* 1984;**18**(4):337–55.
292. Hotchkiss KM., Clark NM., Olivares-Navarrete R. Macrophage response to hydrophilic biomaterials regulates MSC recruitment and T-helper cell populations. *Biomaterials* 2018;**182**:202–15. Doi: 10.1016/j.biomaterials.2018.08.029.
 293. Gittens RA., Olivares-Navarrete R., Cheng A., Anderson DM., McLachlan T., Stephan I., et al. The roles of titanium surface micro/nanotopography and wettability on the differential response of human osteoblast lineage cells. *Acta Biomater* 2013;**9**(4):6268–77. Doi: 10.1016/j.actbio.2012.12.002 [doi].
 294. Lotz EM., Olivares-Navarrete R., Berner S., Boyan BD., Schwartz Z. Osteogenic response of human MSCs and osteoblasts to hydrophilic and hydrophobic nanostructured titanium implant surfaces. *J Biomed Mater Res A* 2016;**104**(12):3137–48. Doi: 10.1002/jbm.a.35852.
 295. Buser D., Broggini N., Wieland M., Schenk RK., Denzer AJ., Cochran DL., et al. Enhanced bone apposition to a chemically modified SLA titanium surface. *J Dent Res* 2004;**83**(7):529–33. Doi: 10.1177/154405910408300704.
 296. Ferguson SJ., Broggini N., Wieland M., de Wild M., Rupp F., Geis-Gerstorfer J., et al. Biomechanical evaluation of the interfacial strength of a chemically modified sandblasted and acid-etched titanium surface. *J Biomed Mater Res A* 2006;**78**(2):291–7. Doi: 10.1002/jbm.a.30678 [doi].
 297. Schwarz F., Herten M., Sager M., Wieland M., Dard M., Becker J. Histological and immunohistochemical analysis of initial and early osseous integration at chemically modified and conventional SLA titanium implants: preliminary results of a pilot study in dogs. *Clin Oral Implants Res* 2007;**18**(4):481–8. Doi: 10.1111/j.1600-0501.2007.01341.x.
 298. Schwarz F., Ferrari D., Herten M., Mihatovic I., Wieland M., Sager M., et al. Effects of surface hydrophilicity and microtopography on early stages of soft and hard tissue integration at non-submerged titanium implants: an immunohistochemical study in dogs. *J Periodontol* 2007;**78**(11):2171–84. Doi: 10.1902/jop.2007.070157.
 299. Schwarz F., Wieland M., Schwartz Z., Zhao G., Rupp F., Geis-Gerstorfer J., et al. Potential of chemically modified hydrophilic surface characteristics to support tissue integration of titanium dental implants. *J Biomed Mater Res B Appl Biomater* 2009;**88**(2):544–57. Doi: 10.1002/jbm.b.31233.
 300. Nakanishi K., Sakiyama T., Imamura K. On the adsorption of proteins on solid surfaces, a common but very complicated phenomenon. *J Biosci Bioeng* 2001. Doi: 10.1016/S1389-

- 1723(01)80127-4.
301. Att W., Hori N., Iwasa F., Yamada M., Ueno T., Ogawa T. The effect of UV-photofunctionalization on the time-related bioactivity of titanium and chromium-cobalt alloys. *Biomaterials* 2009;**30**(26):4268–76. Doi: 10.1016/j.biomaterials.2009.04.048.
 302. Hayashi R., Ueno T., Migita S., Tsutsumi Y., Doi H., Ogawa T., et al. Hydrocarbon Deposition Attenuates Osteoblast Activity on Titanium. *J Dent Res* 2014;**93**(7):698–703. Doi: 10.1177/0022034514536578.
 303. Jimbo R., Coelho PG., Bryington M., Baldassarri M., Tovar N., Currie F., et al. Nano hydroxyapatite-coated implants improve bone nanomechanical properties. *J Dent Res* 2012;**91**(12):1172–7. Doi: 10.1177/0022034512463240 [doi].
 304. Sul Y-T., Byon E., Jeong Y. Biomechanical measurements of calcium-incorporated oxidized implants in rabbit bone: effect of calcium surface chemistry of a novel implant. *Clin Implant Dent Relat Res* 2004;**6**(2):101–10.
 305. Mendonça G., Mendonça DBS., Simões LGP., Araújo AL., Leite ER., Duarte WR., et al. Nanostructured alumina-coated implant surface: effect on osteoblast-related gene expression and bone-to-implant contact in vivo. *Int J Oral Maxillofac Implants* 2009;**24**(2):205–15.
 306. Bougas K., Jimbo R., Vandeweghe S., Hayashi M., Bryington M., Kozai Y., et al. Bone apposition to laminin-1 coated implants: histologic and 3D evaluation. *Int J Oral Maxillofac Surg* 2013;**42**(5):677–82. Doi: 10.1016/j.ijom.2012.11.008 [doi].
 307. Coelho PG., Cardaropoli G., Suzuki M., Lemons JE. Early healing of nanothickness bioceramic coatings on dental implants. An experimental study in dogs. *J Biomed Mater Res B, Appl Biomater* 2009;**88**(2):387–93. Doi: 10.1002/jbm.b.31090 [doi].
 308. Coelho PG., Granjeiro JM., Romanos GE., Suzuki M., Silva NR., Cardaropoli G., et al. Basic research methods and current trends of dental implant surfaces. *J Biomed Mater Res B, Appl Biomater* 2009;**88**(2):579–96. Doi: 10.1002/jbm.b.31264 [doi].
 309. Faeda RS., Tavares HS., Sartori R., Guastaldi AC., Marcantonio E. Biological performance of chemical hydroxyapatite coating associated with implant surface modification by laser beam: biomechanical study in rabbit tibias. *J Oral Maxillofac Surg Off J Am Assoc Oral Maxillofac Surg* 2009;**67**(8):1706–15. Doi: 10.1016/j.joms.2009.03.046.

310. Faeda RS., Spin-Neto R., Marcantonio E., Guastaldi AC., Marcantonio Jr. E. Laser ablation in titanium implants followed by biomimetic hydroxyapatite coating: Histomorphometric study in rabbits. *Microsc Res Tech* 2012;**75**(7):940–8. Doi: 10.1002/jemt.22018.
311. Klokkevold PR., Nishimura RD., Adachi M., Caputo A. Osseointegration enhanced by chemical etching of the titanium surface: A torque removal study in the rabbit. *Clin Oral Implants Res* 1997. Doi: 10.1034/j.1600-0501.1997.080601.x.
312. Sartoretto SC., Alves ATNN., Resende RFB., Calasans-Maia J., Granjeiro JM., Calasans-Maia MD. Early osseointegration driven by the surface chemistry and wettability of dental implants. *J Appl Oral Sci* 2015;**23**(3):279–87. Doi: 10.1590/1678-775720140483.
313. Velázquez-Cayón R., Castillo-Dalí G., Corcuera-Flores J-R., Serrera-Figallo M-A., Castillo-Oyagüe R., González-Martín M., et al. Production of bone mineral material and BMP-2 in osteoblasts cultured on double acid-etched titanium. *Med Oral Patol Oral Cir Bucal* 2017;**22**(5):e659. Doi: 10.4317/medoral.22071.
314. Jinno Y., Jimbo R., Tovar N., Teixeira HS., Witek L., Coelho PG. In Vivo Evaluation of Dual Acid-Etched and Grit-Blasted/Acid-Etched Implants With Identical Macrogeometry in High-Density Bone. *Implant Dent* 2017. Doi: 10.1097/ID.0000000000000672.
315. Calvo-Guirado JL., Satorres M., Negri B., Ramirez-Fernandez P., Maté-Sánchez de Val JE., Maté-Sánchez JE., et al. Biomechanical and histological evaluation of four different titanium implant surface modifications: an experimental study in the rabbit tibia. *Clin Oral Investig* 2014;**18**(5):1495–505. Doi: 10.1007/s00784-013-1120-2.
316. Guo J., Padilla RJ., Ambrose W., De Kok IJ., Cooper LF. The effect of hydrofluoric acid treatment of TiO₂ grit blasted titanium implants on adherent osteoblast gene expression in vitro and in vivo. *Biomaterials* 2007;**28**(36):5418–25. Doi: 10.1016/j.biomaterials.2007.08.032.
317. Ishizawa H., Ogino M. Formation and characterization of anodic titanium oxide films containing Ca and P. *J Biomed Mater Res* 1995;**29**(1):65–72. Doi: 10.1002/jbm.820290110.
318. Sul Y-T., Johansson CB., Jeong Y., Wennerberg A., Albrektsson T. Resonance frequency and removal torque analysis of implants with turned and anodized surface oxides. *Clin Oral Implants Res* 2002;**13**(3):252–9.
319. El-wassefy NA., Hammouda IM., Habib ANEA., El-awady

- GY., Marzook HA. Assessment of anodized titanium implants bioactivity. *Clin Oral Implants Res* 2014;**25**(2):1. Doi: 10.1111/clr.12031.
320. Coelho PG., Lemons JE. Physico/chemical characterization and in vivo evaluation of nanothickness bioceramic depositions on alumina-blasted/acid-etched Ti-6Al-4V implant surfaces. *J Biomed Mater Res A* 2009;**90**(2):351–61. Doi: 10.1002/jbm.a.32097.
 321. Vercaigne S., Wolke JG., Naert I., Jansen JA. A mechanical evaluation of TiO₂-gritblasted and Ca-P magnetron sputter coated implants placed into the trabecular bone of the goat: Part 1. *Clin Oral Implants Res* 2000;**11**(4):305–13.
 322. Elias CN., Meirelles L. Improving osseointegration of dental implants. *Expert Rev Med Devices* 2010;**7**(2):241–56. Doi: 10.1586/erd.09.74 [doi].
 323. Cochran DL., Nummikoski P V., Higginbottom FL., Hermann JS., Makins SR., Buser D. Evaluation of an endosseous titanium implant with a sandblasted and acid-etched surface in the canine mandible: radiographic results. *Clin Oral Implants Res* 1996;**7**(3):240–52.
 324. Al-Nawas B., Groetz KA., Goetz H., Duschner H., Wagner W. Comparative histomorphometry and resonance frequency analysis of implants with moderately rough surfaces in a loaded animal model. *Clin Oral Implants Res* 2008;**19**(1):1–8. Doi: CLR1396 [pii].
 325. Cochran DL., Schenk RK., Lussi A., Higginbottom FL., Buser D. Bone response to unloaded and loaded titanium implants with a sandblasted and acid-etched surface: a histometric study in the canine mandible. *J Biomed Mater Res* 1998;**40**(1):1–11. Doi: AID-JBM1>3.0.CO;2-Q [pii].
 326. Quinlan P., Nummikoski P., Schenk R., Cagna D., Mellonig J., Higginbottom F., et al. Immediate and early loading of SLA ITI single-tooth implants: an in vivo study. *Int J Oral Maxillofac Implants* 2005;**20**(3):360–70.
 327. Chambrone L., Shibli JA., Mercurio CE., Cardoso B., Preshaw PM. Efficacy of standard (SLA) and modified sandblasted and acid-etched (SLActive) dental implants in promoting immediate and/or early occlusal loading protocols: a systematic review of prospective studies. *Clin Oral Implants Res* 2015;**26**(4):359–70. Doi: 10.1111/clr.12347 [doi].
 328. Kim YK., Kim JH., Yi YJ., Kwon MJ., Yun PY. Prospective Comparative Study of Tapered Implants with SLA Surfaces in the Maxillary Posterior Area According to 3- and 6-Month Loading Time. *Int J Periodontics Restorative Dent*

- 2015;**35**(2):271–6. Doi: 10.11607/prd.1789 [doi].
329. Khang W., Feldman S., Hawley CE., Gunsolley J. A multi-center study comparing dual acid-etched and machined-surfaced implants in various bone qualities. *J Periodontol* 2001;**72**(10):1384–90. Doi: 10.1902/jop.2001.72.10.1384 [doi].
 330. Derks J., Håkansson J., Wennström JL., Tomasi C., Larsson M., Berglundh T. Effectiveness of implant therapy analyzed in a Swedish population: early and late implant loss. *J Dent Res* 2015;**94**(3 Suppl):51S. Doi: 10.1177/0022034514563077.
 331. Bowers M., Yoo D., Marin C., Gil L., Shabaka N., Goldstein M., et al. Surface characterization and in vivo evaluation of laser sintered and machined implants followed by resorbable-blasting media process: A study in sheep. *Med Oral Patol Oral Cir Bucal* 2016:e213. Doi: 10.4317/medoral.20946.
 332. Shen J., Liu J., Chen X., Wang X., He F., Wang H. The In Vivo Bone Response of Ultraviolet-Irradiated Titanium Implants Modified with Spontaneously Formed Nanostructures: An Experimental Study in Rabbits. *Int J Oral Maxillofac Implants* 2016;**31**(4):776–84. Doi: 10.11607/jomi.4309.
 333. Svanborg LM., Hoffman M., Andersson M., Currie F., Kjellin P., Wennerberg A. The effect of hydroxyapatite nanocrystals on early bone formation surrounding dental implants. *Int J Oral Maxillofac Surg* 2011;**40**(3):308–15. Doi: 10.1016/j.ijom.2010.10.010 [doi].
 334. Bougas K. *On the influence of biochemical coating on implant bone incorporation*. Malmö University, 2012.
 335. Cecchinato F. *On magnesium-modified titanium coatings and magnesium alloys for oral orthopaedic applications: in vitro investigation*. Malmö University, 2015.
 336. Johansson P. *On hydroxyapatite modified peek implants for bone applications*. Malmö university, 2017.
 337. Alenezi A. *On enhancement of bone formation using local drug delivery systems*. Malmö university, 2018.
 338. Galli S. *On magnesium-containing implants for bone applications*. Malmö university, 2016.
 339. Vetrone F., Variola F., Tambasco de Oliveira P., Zalzal SF., Yi J-H., Sam J., et al. Nanoscale oxidative patterning of metallic surfaces to modulate cell activity and fate. *Nano Lett* 2009;**9**(2):659–65. Doi: 10.1021/nl803051f.
 340. Cipriano AF., Lin J., Miller C., Lin A., Cortez Alcaraz MC., Soria P., et al. Anodization of magnesium for biomedical applications – Processing, characterization, degradation and

- cytocompatibility. *Acta Biomater* 2017. Doi: 10.1016/j.actbio.2017.08.017.
341. Fröjd V., Franke-Stenport V., Meirelles L., Wennerberg A. Increased bone contact to a calcium-incorporated oxidized commercially pure titanium implant: an in-vivo study in rabbits. *Int J Oral Maxillofac Surg* 2008;**37**(6):561–6. Doi: 10.1016/j.ijom.2008.01.020.
 342. Tugulu S., Löwe K., Scharnweber D., Schlottig F. Preparation of superhydrophilic microrough titanium implant surfaces by alkali treatment. *J Mater Sci Mater Med* 2010;**21**(10):2751–63.
 343. Giro G., Tovar N., Witek L., Marin C., Silva NRF., Bonfante EA., et al. Osseointegration assessment of chairside argon-based nonthermal plasma-treated Ca-P coated dental implants 2013;**101**(1):98–103.
 344. Canullo L., Genova T., Tallarico M., Gautier G., Mussano F., Botticelli D. Plasma of Argon Affects the Earliest Biological Response of Different Implant Surfaces: An In Vitro Comparative Study. *J Dent Res* 2016;**95**(5):566–73. Doi: 10.1177/0022034516629119.
 345. Park JH., Olivares-Navarrete R., Wasilewski CE., Boyan BD., Tannenbaum R., Schwartz Z. Use of polyelectrolyte thin films to modulate osteoblast response to microstructured titanium surfaces. *Biomaterials* 2012;**33**(21):5267–77. Doi: 10.1016/j.biomaterials.2012.03.074.
 346. Sawase T., Jimbo R., Baba K., Shibata Y., Ikeda T., Atsuta M. Photo-induced hydrophilicity enhances initial cell behavior and early bone apposition 2008;**19**(5):491–6. Doi: 10.1111/j.1600-0501.2007.01509.x.
 347. Liang L., Krieg P., Rupp F., Kammerle-Müller E., Spintzyk S., Richter M., et al. Osteoblast Response to Different UVA-Activated Anatase Implant Coatings. *Adv Mater Interfaces* 2018:1–13.
 348. Rupp F., Liang L., Geis-Gerstorfer J., Scheideler L., Hüttig F. Surface characteristics of dental implants: A review. *Dent Mater Off Publ Acad Dent Mater* 2018;**34**(1):40–57. Doi: 10.1016/j.dental.2017.09.007.
 349. Giljean S., Bigerellem M., Anselme K., Haidara H. New insights on contact angle/roughness dependence on high surface energy materials. *Appl Surf Sci* 2011;**257**(22):9631–8.
 350. Boyan B., Lotz EM., Schwartz Z. Roughness and Hydrophilicity as Osteogenic Biomimetic Surface Properties. *Tissue Eng Part A* 2017. Doi: 10.1089/ten.TEA.2017.0048.
 351. Wennerberg A., Svanborg LM., Berner S., Andersson M.

- Spontaneously formed nanostructures on titanium surfaces. *Clin Oral Implants Res* 2013;**24**(2):203–9. Doi: 10.1111/j.1600-0501.2012.02429.x [doi].
352. Lai HC., Zhuang LF., Liu X., Wieland M., Zhang ZY., Zhang ZY. The influence of surface energy on early adherent events of osteoblast on titanium substrates. *J Biomed Mater Res A* 2010;**93**(1):289–96. Doi: 10.1002/jbm.a.32542 [doi].
 353. Raines AL., Olivares-Navarrete R., Wieland M., Cochran DL., Schwartz Z., Boyan BD. Regulation of angiogenesis during osseointegration by titanium surface microstructure and energy. *Biomaterials* 2010;**31**(18):4909–17. Doi: 10.1016/j.biomaterials.2010.02.071.
 354. An N., Schedle A., Wieland M., Andrukhov O., Matejka M., Rausch-Fan X. Proliferation, behavior, and cytokine gene expression of human umbilical vascular endothelial cells in response to different titanium surfaces. *J Biomed Mater Res A* 2010;**93**(1):364–72. Doi: 10.1002/jbm.a.32539.
 355. Raines AL., Berger MB., Patel N., Hyzy SL., Boyan BD., Schwartz Z. VEGF-A regulates angiogenesis during osseointegration of Ti implants via paracrine/autocrine regulation of osteoblast response to hierarchical microstructure of the surface. *J Biomed Mater Res A* 2019;**107**(2):423–33. Doi: 10.1002/jbm.a.36559.
 356. Hyzy SL., Olivares-Navarrete R., Hutton DL., Tan C., Boyan BD., Schwartz Z. Microstructured titanium regulates interleukin production by osteoblasts, an effect modulated by exogenous BMP-2. *Acta Biomater* 2013;**9**(3):5821. Doi: 10.1016/j.actbio.2012.10.030.
 357. Hamlet S., Alfarsi M., George R., Ivanovski S. The effect of hydrophilic titanium surface modification on macrophage inflammatory cytokine gene expression. *Clin Oral Implants Res* 2012;**23**(5):584–90. Doi: 10.1111/j.1600-0501.2011.02325.x [doi].
 358. Wall I., Donos N., Carlqvist K., Jones F., Brett P. Modified titanium surfaces promote accelerated osteogenic differentiation of mesenchymal stromal cells in vitro. *Bone* 2009;**45**(1):17–26. Doi: 10.1016/j.bone.2009.03.662.
 359. Rosa AL., Kato RB., Castro Raucci LMS., Teixeira LN., de Oliveira FS., Bellesini LS., et al. Nanotopography drives stem cell fate toward osteoblast differentiation through $\alpha 1\beta 1$ integrin signaling pathway. *J Cell Biochem* 2014;**115**(3):540–8. Doi: 10.1002/jcb.24688.
 360. Yin L., Chang Y., You Y., Liu C., Li J., Lai HC. Biological responses of human bone mesenchymal stem cells to Ti and

- TiZr implant materials. *Clin Implant Dent Relat Res* 2019. Doi: 10.1111/cid.12756.
361. Mamalis AA., Markopoulou C., Vrotsos I., Koutsilieri M. Chemical modification of an implant surface increases osteogenesis and simultaneously reduces osteoclastogenesis: an in vitro study. *Clin Oral Implants Res* 2011;**22**(6):619–26. Doi: 10.1111/j.1600-0501.2010.02027.x.
 362. Bang SM., Moon HJ., Kwon YD., Yoo JY., Pae A., Kwon IK. Osteoblastic and osteoclastic differentiation on SLA and hydrophilic modified SLA titanium surfaces. *Clin Oral Implants Res* 2014;**25**(7):831–7. Doi: 10.1111/clr.12146 [doi].
 363. Lotz EM., Berger MB., Schwartz Z., Boyan BD. Regulation of osteoclasts by osteoblast lineage cells depends on titanium implant surface properties. *Acta Biomater* 2018. Doi: 10.1016/j.actbio.2017.12.039.
 364. Pinotti FE., de Oliveira GJPL., Aroni MAT., Marcantonio RAC., Marcantonio E. Analysis of osseointegration of implants with hydrophilic surfaces in grafted areas: A Preclinical study. *Clin Oral Implants Res* 2018;**29**(10):963–72. Doi: 10.1111/clr.13361.
 365. Gottlow J., Barkarm S., Sennerby L. An experimental comparison of two different clinically used implant designs and surfaces. *Clin Implant Dent Relat Res* 2012;**14** Suppl 1:204. Doi: 10.1111/j.1708-8208.2012.00439.x.
 366. Vasak C., Busenlechner D., Schwarze UY., Leitner HF., Munoz Guzon F., Hefti T., et al. Early bone apposition to hydrophilic and hydrophobic titanium implant surfaces: a histologic and histomorphometric study in minipigs. *Clin Oral Implants Res* 2014;**25**(12):1378–85. Doi: 10.1111/clr.12277 [doi].
 367. Guler AU., Sumer M., Duran I., Sandikci EO., Telcioglu NT. Resonance frequency analysis of 208 Straumann dental implants during the healing period. *J Oral Implantol* 2013;**39**(2):161–7. Doi: 10.1563/AAID-JOI-D-11-00060 [doi].
 368. Wennerberg A., Galli S., Albrektsson T. Current knowledge about the hydrophilic and nanostructured SLActive surface. *Clin Cosmet Investig Dent* 2011;**3**:59–67. Doi: 10.2147/CCIDE.S15949.
 369. Coelho PG., Jimbo R. Osseointegration of metallic devices: Current trends based on implant hardware design. *Arch Biochem Biophys* 2014;**561**:99–108. Doi: 10.1016/j.abb.2014.06.033.
 370. Falisi G., Severino M., Rastelli C., Bernardi S., Caruso S., Galli M., et al. The effects of surgical preparation techniques and implant macro-geometry on primary stability: An in vitro

- study. *Med Oral Patol Oral Cir Bucal* 2017;**22**(2):e206.
371. Elias CN., Rocha FA., Nascimento AL., Coelho PG., Elias CN., Rocha FA., et al. Influence of implant shape, surface morphology, surgical technique and bone quality on the primary stability of dental implants. *J Mech Behav Biomed Mater* 2012;**16**:169–80. Doi: 10.1016/j.jmbbm.2012.10.010.
 372. Lan T-H., Du J-K., Pan C-Y., Lee H-E., Chung W-H. Biomechanical analysis of alveolar bone stress around implants with different thread designs and pitches in the mandibular molar area. *Clin Oral Investig* 2012;**16**(2):363–9. Doi: 10.1007/s00784-011-0517-z.
 373. Chowdhary R., Jimbo R., Thomsen C., Carlsson L., Wennerberg A. Biomechanical evaluation of macro and micro designed screw-type implants: an insertion torque and removal torque study in rabbits. *Clin Oral Implants Res* 2013;**24**(3):342–6. Doi: 10.1111/j.1600-0501.2011.02336.x.
 374. Shunmugasamy VC., Gupta N., Pessoa RS., Janal MN., Coelho PG. Influence of clinically relevant factors on the immediate biomechanical surrounding for a series of dental implant designs. *J Biomech Eng* 2011;**133**(3):031005. Doi: 10.1115/1.4003318 [doi].
 375. Gil LF., Suzuki M., Janal MN., Tovar N., Marin C., Granato R., et al. Progressive plateau root form dental implant osseointegration: A human retrieval study. *J Biomed Mater Res B Appl Biomater* 2015;**103**(6):1328–32. Doi: 10.1002/jbm.b.33311.
 376. Baldassarri M., Bonfante E., Suzuki M., Marin C., Granato R., Tovar N., et al. Mechanical properties of human bone surrounding plateau root form implants retrieved after 0.3-24 years of function. *J Biomed Mater Res B, Appl Biomater* 2012;**100**(7):2015–21. Doi: 10.1002/jbm.b.32786 [doi].
 377. Coelho PG., Suzuki M., Marin C., Granato R., Gil LF., Tovar N., et al. Osseointegration of Plateau Root Form Implants: Unique Healing Pathway Leading to Haversian-Like Long-Term Morphology. *Adv Exp Med Biol* 2015;**881**:111–28. Doi: 10.1007/978-3-319-22345-2_7.
 378. Leonard G., Coelho P., Polyzois I., Stassen L., Claffey N. A study of the bone healing kinetics of plateau versus screw root design titanium dental implants. *Clin Oral Implants Res* 2009;**20**(3):232–9. Doi: 10.1111/j.1600-0501.2008.01640.x.
 379. Coelho PG., Suzuki M., Guimaraes MVM., Marin C., Granato R., Gil JN., et al. Early bone healing around different implant bulk designs and surgical techniques: A study in dogs. *Clin Implant Dent Relat Res* 2010;**12**(3):202–8. Doi:

- 10.1111/j.1708-8208.2009.00153.x.
380. Lioubavina-Hack N., Lang NP., Karring T. Significance of primary stability for osseointegration of dental implants. *Clin Oral Implants Res* 2006;**17**(3):244–50. Doi: CLR1201 [pii].
 381. Norton M. Primary stability versus viable constraint--a need to redefine. *Int J Oral Maxillofac Implants* 2013;**28**(1):19–21.
 382. Ottoni JMP., Oliveira ZFL., Mansini R., Cabral AM. Correlation between placement torque and survival of single-tooth implants. *Int J Oral Maxillofac Implant* 2005;**20**(5):769–76.
 383. Norton MR. The influence of insertion torque on the survival of immediately placed and restored single-tooth implants. *Int J Oral Maxillofac Implant* 2011;**26**(6):1333–43.
 384. Campos., Jimbo R., Bonfante EA., Barbosa DZ., Oliveira MTF., Janal MN., et al. Are insertion torque and early osseointegration proportional? A histologic evaluation. *Clin Oral Implants Res* 2015;**26**(11):1256–60. Doi: 10.1111/clr.12448.
 385. Akça K., Kökat AM., Cömert A., Akkocaoğlu M., Tekdemir I., Cehreli MC. Torque-fitting and resonance frequency analyses of implants in conventional sockets versus controlled bone defects in vitro. *Int J Oral Maxillofac Surg* 2010;**39**(2):169–73. Doi: 10.1016/j.ijom.2009.11.019.
 386. Freitas AC., Bonfante EA., Giro G., Janal MN., Coelho PG. The effect of implant design on insertion torque and immediate micromotion. *Clin Oral Implants Res* 2012;**23**(1):113–8. Doi: 10.1111/j.1600-0501.2010.02142.x.
 387. Degidi M., Dapri G., Piattelli A. Influence of underpreparation on primary stability of implants inserted in poor quality bone sites: an in vitro study. *J Oral Maxillofac Surg* 2015;**73**(6):1084–8. Doi: 10.1016/j.joms.2015.01.029 [doi].
 388. Khayat PG., Arnal HM., Tourbah BI., Sennerby L. Clinical outcome of dental implants placed with high insertion torques (up to 176 Ncm). *Clin Implant Dent Relat Res* 2013;**15**(2):227–33. Doi: 10.1111/j.1708-8208.2011.00351.x [doi].
 389. Rabel A., Kohler SG., Schmidt-Westhausen AM. Clinical study on the primary stability of two dental implant systems with resonance frequency analysis. *Clin Oral Investig* 2007;**11**(3):257–65. Doi: 10.1007/s00784-007-0115-2 [doi].
 390. Romero-Ruiz MM., Gil-Mur FJ., Ríos-Santos JV., Lázaro-Calvo P., Ríos-Carrasco B., Herrero-Climent M. Influence of a Novel Surface of Bioactive Implants on Osseointegration: A

- Comparative and Histomorfometric Correlation and Implant Stability Study in Minipigs. *Int J Mol Sci* 2019. Doi: 10.3390/ijms20092307.
391. Consolo U., Travaglini D., Todisco M., Trisi P., Galli S. Histologic and biomechanical evaluation of the effects of implant insertion torque on peri-implant bone healing. *J Craniofac Surg* 2013;**24**(3):860–5. Doi: 10.1097/SCS.0b013e31827ca3cf.
 392. Halldin A., Jimbo R., Johansson CB., Wennerberg A., Jacobsson M., Albrektsson T., et al. The effect of static bone strain on implant stability and bone remodeling. *Bone* 2011;**49**(4):783–9.
 393. Halldin A., Jimbo R., Johansson CB., Wennerberg A., Jacobsson M., Albrektsson T., et al. Implant stability and bone remodeling after 3 and 13 days of implantation with an initial static strain. *Clin Implant Dent Relat Res* 2014;**16**(3):383–93. Doi: 10.1111/cid.12000 [doi].
 394. Lemons JE. Biomaterials, biomechanics, tissue healing, and immediate-function dental implants. *J Oral Implantol* 2004;**30**(5):318–24. Doi: 10.1563/0712.1 [doi].
 395. Szmukler-Moncler S., Salama H., Reingewirtz Y., Dubruille JH. Timing of loading and effect of micromotion on bone-dental implant interface: review of experimental literature. *J Biomed Mater Res* 1998;**43**(2):192–203. Doi: 10.1002/(SICI)1097-4636(199822)43:2<192::AID-JBM14>3.0.CO;2-K [pii].
 396. Javed F., Ahmed HB., Crespi R., Romanos GE. Role of primary stability for successful osseointegration of dental implants: Factors of influence and evaluation. *Interv Med Appl Sci* 2013;**5**(4):162–7. Doi: 10.1556/IMAS.5.2013.4.3.
 397. Trisi P., Berardini M., Falco A., Podaliri Vulpiani M. Validation of value of actual micromotion as a direct measure of implant micromobility after healing (secondary implant stability). An in vivo histologic and biomechanical study. *Clin Oral Implants Res* 2016;**27**(11):1423–30. Doi: 10.1111/clr.12756.
 398. Eom T-G., Kim H-W., Jeon G-R., Yun M-J., Huh J-B., Jeong C-M. Effects of Different Implant Osteotomy Preparation Sizes on Implant Stability and Bone Response in the Minipig Mandible. *Int J Oral Maxillofac Implants* 2016;**31**(5):997–1006.
 399. Rea M., Lang NP., Ricci S., Mintrone F., González González G., Botticelli D. Healing of implants installed in over- or under-prepared sites--an experimental study in dogs. *Clin Oral*

- Implants Res* 2015;**26**(4):442–6. Doi: 10.1111/clr.12390.
400. Menicucci G., Pachie E., Lorenzetti M., Migliaretti G., Carossa S. Comparison of primary stability of straight-walled and tapered implants using an insertion torque device. *Int J Prosthodont* 2012. Doi: 10.1007/BF03217427.
 401. Baires-Campos., Jimbo R., Bonfante EA., Fonseca-Oliveira MT., Moura C., Zanetta-Barbosa D., et al. Drilling dimension effects in early stages of osseointegration and implant stability in a canine model. *Med Oral Patol Oral Cir Bucal* 2015;**20**(4):471. Doi: 20557 [pii].
 402. Frisardi G., Barone S., Razionale A V., Paoli A., Frisardi F., Tullio A., et al. Biomechanics of the press-fit phenomenon in dental implantology: an image-based finite element analysis. *Head Face Med* 2012;**8**(1):18. Doi: 10.1186/1746-160X-8-18.
 403. Widmark G., Friberg B., Johansson B., Sindet-Pedersen S., Taylor A. Mk III: a third generation of the self-tapping Branemark System implant, including the new Stargrip internal grip design. A 1-year prospective four-center study. *Clin Implant Dent Relat Res* 2003;**5**(4):273–9.
 404. Friberg B., Jisander S., Widmark G., Lundgren A., Ivanoff CJ., Sennerby L., et al. One-year prospective three-center study comparing the outcome of a “soft bone implant” (prototype Mk IV) and the standard Branemark implant. *Clin Implant Dent Relat Res* 2003;**5**(2):71–7.
 405. Alves CC., Neves M. Tapered implants: from indications to advantages 2009;**29**(2):161–7.
 406. Valente ML da C., de Castro DT., Shimano AC., Lepri CP., dos Reis AC. Analysis of the influence of implant shape on primary stability using the correlation of multiple methods. *Clin Oral Investig* 2015;**19**(8):1861–6. Doi: 10.1007/s00784-015-1417-4.
 407. Quirynen M., Naert I., Van Steenberghe D. Fixture design and overload influence marginal bone loss and future success in the Brånemark® system. *Clin Oral Implants Res* 1992;**3**(3):104–11. Doi: 10.1034/j.1600-0501.1992.030302.x.
 408. Payne AGT., Tawse-Smith A., Thomson WM., Duncan WD., Kumara R. One-stage surgery and early loading of three implants for maxillary overdentures: A 1-year report. *Clin Implant Dent Relat Res* 2004. Doi: 10.1111/j.1708-8208.2004.tb00028.x.
 409. Lang NP., Tonetti MS., Suvan JE., Bernard JP., Botticelli D., Fourmouis I., et al. Immediate implant placement with transmucosal healing in areas of aesthetic priority: A multicentre randomized-controlled clinical trial I. Surgical

- outcomes. *Clin Oral Implants Res* 2007. Doi: 10.1111/j.1600-0501.2006.01371.x.
410. Akoglu B., Ucankale M., Ozkan Y., Kulak-Ozkan Y. Five-year treatment outcomes with three brands of implants supporting mandibular overdentures. *Int J Oral Maxillofac Implants* 2011. Doi: 10.1007/978-1-4939-0709-0.
 411. Eser A., Tonuk E., Akca K., Cehreli MC. Predicting time-dependent remodeling of bone around immediately loaded dental implants with different designs. *Med Eng Phys* 2010. Doi: 10.1016/j.medengphy.2009.10.004.
 412. O'Sullivan D., Sennerby L., Jagger D., Meredith N. A comparison of two methods of enhancing implant primary stability. *Clin Implant Dent Relat Res* 2004. Doi: 10.1111/j.1708-8208.2004.tb00027.x.
 413. Cochran D., Stavropoulos A., Obrecht M., Pippenger B., Dard M. A Comparison of Tapered and Nontapered Implants in the Minipig. *Int J Oral Maxillofac Implants* 2016;**31**(6):1341–7.
 414. Balshi TJ., Wolfinger GJ. Two-implant-supported single molar replacement: interdental space requirements and comparison to alternative options. *Int J Periodontics Restorative Dent* 1997.
 415. Thoma DS., Cha J-K., Jung U-W. Treatment concepts for the posterior maxilla and mandible: short implants versus long implants in augmented bone. *J Periodontal Implant Sci* 2017;**47**(1):2. Doi: 10.5051/jpis.2017.47.1.2.
 416. Jung RE., Al-Nawas B., Araujo M., Avila-Ortiz G., Barter S., Brodala N., et al. Group 1 ITI Consensus Report: The influence of implant length and design and medications on clinical and patient-reported outcomes. *Clin Oral Implants Res* 2018. Doi: 10.1111/clr.13342.
 417. Degidi M., Iezzi G., Perrotti V., Piattelli A. Comparative analysis of immediate functional loading and immediate nonfunctional loading to traditional healing periods: a 5-year follow-up of 550 dental implants. *Clin Implant Dent Relat Res* 2009;**11**(4):257–66. Doi: 10.1111/j.1708-8208.2008.00117.x.
 418. Buser D., Mericske-stern R., Bernard P., Pierre J., Behneke A., Behneke N., et al. Long-term evaluation of non-submerged ITI implants. Part 1: 8-year life table analysis of a prospective multi-center study with 2359 implants. *Clin Oral Implants Res* 1997;**8**(3):161–72.
 419. Sánchez-Garcés MA., Costa-Berenguer X., Gay-Escoda C. Short Implants: A Descriptive Study of 273 Implants. *Clin Implant Dent Relat Res* 2012. Doi: 10.1111/j.1708-8208.2010.00301.x.
 420. Lozano-Carrascal N., Salomó-Coll O., Gilabert-Cerdà M.,

- Farré-Pagés N., Gargallo-Albiol J., Hernández-Alfaro F. Effect of implant macro-design on primary stability: A prospective clinical study. *Med Oral Patol Oral y Cir Bucal* 2016. Doi: 10.4317/medoral.21024.
421. Vandeweghe S., Ackermann A., Bronner J., Hattingh A., Tschakaloff A., De Bruyn H. A Retrospective, Multicenter Study on a Novo Wide-Body Implant for Posterior Regions. *Clin Implant Dent Relat Res* 2012;**14**(2):281–92. Doi: 10.1111/j.1708-8208.2009.00253.x.
 422. Vandeweghe S., Hattingh A., Wennerberg A., De Bruyn H. Surgical Protocol and Short-Term Clinical Outcome of Immediate Placement in Molar Extraction Sockets Using a Wide Body Implant. *J Oral Maxillofac Res* 2011;**2**(1):e1. Doi: 10.5037/jomr.2011.2301.
 423. Hattingh A., Hommeze G., De Bruyn H., Huyghe M., Vandeweghe S. A prospective study on ultra-wide diameter dental implants for immediate molar replacement. *Clin Implant Dent Relat Res* 2018;**20**(6):1009–15. Doi: 10.1111/cid.12666.
 424. Lee C-T., Chen Y-W., Starr JR., Chuang S-K. Survival analysis of wide dental implant: systematic review and meta-analysis. *Clin Oral Implants Res* 2016;**27**(10):1251–64. Doi: 10.1111/clr.12730.
 425. Baggi L., Cappelloni I., Di Girolamo M., Maceri F., Vairo G. The influence of implant diameter and length on stress distribution of osseointegrated implants related to crestal bone geometry: a three-dimensional finite element analysis. *J Prosthet Dent* 2008;**100**(6):422–31. Doi: 10.1016/S0022-3913(08)60259-0 [doi].
 426. Anitua E., Tapia R., Luzuriaga F., Orive G. Influence of implant length, diameter, and geometry on stress distribution: a finite element analysis. *Int J Periodontics Restorative Dent* 2010;**30**(1):89–95.
 427. Schiegnitz E., Al-Nawas B. Narrow-diameter implants: A systematic review and meta-analysis. *Clin Oral Implants Res* 2018;**29**:21–40. Doi: 10.1111/clr.13272.
 428. Renouard F., Nisand D. Impact of implant length and diameter on survival rates. *Clin Oral Implants Res* 2006;**17**(S2):35–51. Doi: 10.1111/j.1600-0501.2006.01349.x.
 429. Winkler S., Morris HF., Ochi S. Implant survival to 36 months as related to length and diameter. *Ann Periodontol* 2000;**5**(1):22–31.
 430. Ting M., Palermo M., Donatelli DP., Gaughan JP., Suzuki JB., Jefferies SR. A meta-analysis on the effect of implant characteristics on the survival of the wide-diameter implant. *Int*

- J Implant Dent* 2015;**1**(1):28. Doi: 10.1186/s40729-015-0030-2.
431. Hingsammer L., Watzek G., Pommer B. The influence of crown-to-implant ratio on marginal bone levels around splinted short dental implants: A radiological and clinical short term analysis. *Clin Implant Dent Relat Res* 2017. Doi: 10.1111/cid.12546.
 432. Chowdhary R., Halldin A., Jimbo R., Wennerberg A. Evaluation of stress pattern generated through various thread designs of dental implants loaded in a condition of immediately after placement and on osseointegration--an FEA study. *Implant Dent* 2013;**22**(1):91–6. Doi: 10.1097/ID.0b013e31827daf55 [doi].
 433. Falco A., Berardini M., Trisi P. Correlation Between Implant Geometry, Implant Surface, Insertion Torque, and Primary Stability: In Vitro Biomechanical Analysis. *Int J Oral Maxillofac Implants* 2018;**33**(4):824–30. Doi: 10.11607/jomi.6285.
 434. Sotto-Maior BS., Rocha EP., de Almeida EO., Freitas-Júnior AC., Anchieta RB., Del Bel Cury AA. Influence of high insertion torque on implant placement: an anisotropic bone stress analysis. *Braz Dent J* 2010;**21**(6):508–14.
 435. Chang PK., Chen YC., Huang CC., Lu WH., Chen YC., Tsai HH. Distribution of micromotion in implants and alveolar bone with different thread profiles in immediate loading: a finite element study. *Int J Oral Maxillofac Implants* 2012;**27**(6):96.
 436. Kim DR., Lim YJ., Kim MJ., Kwon HB., Kim SH. Self-cutting blades and their influence on primary stability of tapered dental implants in a simulated low-density bone model: A laboratory study. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology* 2011. Doi: 10.1016/j.tripleo.2010.12.001.
 437. Kim YS., Lim YJ. Primary stability and self-tapping blades: Biomechanical assessment of dental implants in medium-density bone. *Clin Oral Implants Res* 2011. Doi: 10.1111/j.1600-0501.2010.02089.x.
 438. Nevins M., Nevins ML., Schupbach P., Fiorellini J., Lin Z., Kim DM. The impact of bone compression on bone-to-implant contact of an osseointegrated implant: a canine study. *Int J Periodontics Restor Dent* 2012.
 439. Eraslan O., Inan O. The effect of thread design on stress distribution in a solid screw implant: a 3D finite element analysis. *Clin Oral Investig* 2010;**14**(4):411–6. Doi:

- 10.1007/s00784-009-0305-1.
440. Mosavar A., Ziaei A., Kadkhodaei M. The effect of implant thread design on stress distribution in anisotropic bone with different osseointegration conditions: a finite element analysis. *Int J Oral Maxillofac Implants* 2015;**30**(6):1317–26. Doi: 10.11607/jomi.4091.
 441. Desai SR., Desai MS., Katti G., Karthikeyan I. Evaluation of design parameters of eight dental implant designs: A two-dimensional finite element analysis. *Niger J Clin Pract* 2012. Doi: 10.4103/1119-3077.97308.
 442. Vivan Cardoso M., Vandamme K., Chaudhari A., De Rycker J., Van Meerbeek B., Naert I., et al. Dental Implant Macro-Design Features Can Impact the Dynamics of Osseointegration. *Clin Implant Dent Relat Res* 2015. Doi: 10.1111/cid.12178.
 443. Abuhussein H., Pagni G., Rebaudi A., Wang H-LL. The effect of thread pattern upon implant osseointegration 2010;**21**(2):129–36.
 444. Ma P., Xiong W., Tan B., Geng W., Liu J., Li W., et al. Influence of thread pitch, helix angle, and compactness on micromotion of immediately loaded implants in three types of bone quality: A three-dimensional finite element analysis. *Biomed Res Int* 2014. Doi: 10.1155/2014/983103.
 445. Orsini E., Giavaresi G., Trirè A., Ottani V., Salgarello S. Dental implant thread pitch and its influence on the osseointegration process: an in vivo comparison study. *Int J Oral Maxillofac Implants* 2012;**27**(2):383–92.
 446. Coelho PG., Granato R., Marin C., Teixeira HS., Suzuki M., Valverde GB., et al. The effect of different implant macrogeometries and surface treatment in early biomechanical fixation: an experimental study in dogs. *J Mech Behav Biomed Mater* 2011;**4**(8):1974–81. Doi: 10.1016/j.jmbbm.2011.06.016.
 447. Chowdhary R., Jimbo R., Thomsen CS., Carlsson L., Wennerberg A. The osseointegration stimulatory effect of macrogeometry-modified implants: a study in the rabbit. *Clin Oral Implants Res* 2014;**25**(9):1051–5. Doi: 10.1111/clr.12212.
 448. Chowdhary R., Halldin A., Jimbo R., Wennerberg A. Influence of Micro Threads Alteration on Osseointegration and Primary Stability of Implants: An FEA and In Vivo Analysis in Rabbits. *Clin Implant Dent Relat Res* 2015;**17**(3):562–9. Doi: 10.1111/cid.12143.
 449. Negri B., Calvo Guirado JL., Maté Sánchez de Val JE., Delgado Ruíz RA., Ramírez Fernández MP., Barona Dorado

- C. Peri-implant tissue reactions to immediate nonocclusal loaded implants with different collar design: an experimental study in dogs. *Clin Oral Implants Res* 2014;**25**(2):54. Doi: 10.1111/clr.12047.
450. Pandey RK., Panda SS. Drilling of bone: A comprehensive review. *J Clin Orthop Trauma* 2013. Doi: 10.1016/j.jcot.2013.01.002.
 451. Eriksson AR., Albrektsson T. Temperature threshold levels for heat-induced bone tissue injury: A vital-microscopic study in the rabbit. *J Prosthet Dent* 1983. Doi: 10.1016/0022-3913(83)90174-9.
 452. Harder S., Egert C., Freitag-Wolf S., Mehl C., Kern M. Intraosseous Temperature Changes During Implant Site Preparation: In Vitro Comparison of Thermocouples and Infrared Thermography. *Int J Oral Maxillofac Implants* 2018. Doi: 10.11607/jomi.6222.
 453. Oh H., Kim B., Kim H-Y., Yeo I-S., Wikesjö U., Koo K-T. Implant Drill Characteristics: Thermal and Mechanical Effects of Two-, Three-, and Four-Fluted Drills. *Int J Oral Maxillofac Implants* 2017. Doi: 10.11607/jomi.4819.
 454. Brisman DL. The effect of speed, pressure, and time on bone temperature during the drilling of implant sites. *Int J Oral Maxillofac Implants* 1997. Doi: 10.1016/j.eururo.2006.12.033.
 455. Sharawy M., Misch CE., Weller N., Tehemar S. Heat generation during implant drilling: The significance of motor speed. *J Oral Maxillofac Surg* 2002. Doi: 10.1053/joms.2002.34992.
 456. Gehrke SA., Aramburú Júnior JS., Pérez-Albacete Martínez C., Ramirez Fernandez MP., Maté Sánchez de Val JE., Calvo-Guirado JL. The influence of drill length and irrigation system on heat production during osteotomy preparation for dental implants: an ex vivo study. *Clin Oral Implants Res* 2018. Doi: 10.1111/clr.12827.
 457. Eriksson AR., Albrektsson T., Albrektsson B. Heat caused by drilling cortical bone: Temperature measured in vivo in patients and animals. *Acta Orthop* 1984. Doi: 10.3109/17453678408992410.
 458. Feldmann A., Wili P., Maquer G., Zysset P. The thermal conductivity of cortical and cancellous bone. *Eur Cells Mater* 2018;**35**:25–33. Doi: 10.22203/eCM.v035a03.
 459. Wang S., Lacefield WR., Lemons JE. Interfacial shear strength and histology of plasma sprayed and sintered hydroxyapatite implants in vivo. *Biomaterials* 1996. Doi: 10.1016/0142-

- 9612(96)00020-8.
460. Rohner D., Tay A., Chung SM., Hutmacher DW. Interface of unloaded titanium implants in the iliac crest, fibula, and scapula: a histomorphometric and biomechanical study in the pig. *Int J Oral Maxillofac Implant* 2004.
 461. Eriksson RA., Albrektsson T. The effect of heat on bone regeneration: An experimental study in the rabbit using the bone growth chamber. *J Oral Maxillofac Surg* 1984. Doi: 10.1016/0278-2391(84)90417-8.
 462. Jimbo R., Giro G., Marin C., Granato R., Suzuki M., Tovar N., et al. Simplified Drilling Technique Does Not Decrease Dental Implant Osseointegration: A Preliminary Report. *J Periodontol* 2013;**84**(11):1599–605. Doi: 10.1902/jop.2012.120565.
 463. Jimbo R., Janal MN., Marin C., Giro G., Tovar N., Coelho PG. The effect of implant diameter on osseointegration utilizing simplified drilling protocols. *Clin Oral Implants Res* 2014;**25**(11):1295–300. Doi: 10.1111/clr.12268.
 464. Giro G., Tovar N., Marin C., Bonfante EA., Jimbo R., Suzuki M., et al. The effect of simplifying dental implant drilling sequence on osseointegration: an experimental study in dogs. *Int J Biomater* 2013;**2013**.
 465. Tabassum A., Meijer GJ., Frank Walboomers X., Jansen JA. Biological limits of the undersized surgical technique: A study in goats. *Clin Oral Implants Res* 2011. Doi: 10.1111/j.1600-0501.2010.02016.x.
 466. Jimbo R., Tovar N., Yoo DY., Janal MN., Anchieta RB., Coelho PG. The effect of different surgical drilling procedures on full laser-etched microgrooves surface-treated implants: an experimental study in sheep. *Clin Oral Implants Res* 2014;**25**(9):1072–7. Doi: 10.1111/clr.12216.
 467. Summers RB. A new concept in maxillary implant surgery: the osteotome technique. *Compendium* 1994. Doi: 10.1109/CCGrid.2012.129.
 468. Buchter A., Kleinheinz J., Wiesmann HP., Jayaranan M., Joos U., Meyer U. Interface reaction at dental implants inserted in condensed bone. *Clin Oral Implants Res* 2005;**16**(5):509–17. Doi: 10.1111/j.1600-0501.2005.01111.x.
 469. Wang L., Wu Y., Perez KC., Hyman S., Brunski JB., Tulu U., et al. Effects of Condensation on Peri-implant Bone Density and Remodeling. *J Dent Res* 2017. Doi: 10.1177/0022034516683932.
 470. S Huwais - US Patent App. 13/608 307., 2013 undefined. Fluted osteotome and surgical method for use. *Google Patents*

- n.d.
471. Huwais S. Autografting osteotome, 2014.
 472. Huwais S., Meyer E. A Novel Osseous Densification Approach in Implant Osteotomy Preparation to Increase Biomechanical Primary Stability, Bone Mineral Density, and Bone-to-Implant Contact. *Int J Oral Maxillofac Implants* 2016. Doi: 10.11607/jomi.4817.
 473. Jimbo R., Tovar N., Marin C., Teixeira HS., Anchieta RB., Silveira LM., et al. The impact of a modified cutting flute implant design on osseointegration. *Int J Oral Maxillofac Surg* 2014. Doi: 10.1016/j.ijom.2014.01.016.
 474. Trisi P., Berardini M., Falco A., Podaliri Vulpiani M. New osseodensification implant site preparation method to increase bone density in low-density bone: In vivo evaluation in sheep. *Implant Dent* 2016. Doi: 10.1097/ID.0000000000000358.
 475. Lahens B., Neiva R., Tovar N., Alifarag AM., Jimbo R., Bonfante EA., et al. Biomechanical and histologic basis of osseodensification drilling for endosteal implant placement in low density bone. An experimental study in sheep. *J Mech Behav Biomed Mater* 2016. Doi: 10.1016/j.jmbbm.2016.06.007.
 476. Oliveira PGFP de., Bergamo ETP., Neiva R., Bonfante EA., Witek L., Tovar N., et al. Osseodensification outperforms conventional implant subtractive instrumentation: A study in sheep. *Mater Sci Eng C* 2018. Doi: 10.1016/j.msec.2018.04.051.
 477. Witek L., Neiva R., Alifarag A., Shahraki F., Sayah G., Tovar N., et al. Absence of Healing Impairment in Osteotomies Prepared via Osseodensification Drilling. *Int J Periodontics Restorative Dent* 2019;39(1):65–71. Doi: 10.11607/prd.3504.
 478. Lahens B., Lopez CD., Neiva RF., Bowers MM., Jimbo R., Bonfante EA., et al. The effect of osseodensification drilling for endosteal implants with different surface treatments: A study in sheep. *J Biomed Mater Res - Part B Appl Biomater* 2018. Doi: 10.1002/jbm.b.34154.
 479. Friberg B., Sennerby L., Gröndahl K., Bergström C., Bäck T., Lekholm U. On cutting torque measurements during implant placement: a 3-year clinical prospective study. *Clin Implant Dent Relat Res* 1999;1(2):75–83.
 480. Turkyilmaz I., Aksoy U., McGlumphy EA. Two alternative surgical techniques for enhancing primary implant stability in the posterior maxilla: A clinical study including bone density, insertion torque, and resonance frequency analysis data. *Clin Implant Dent Relat Res* 2008. Doi: 10.1111/j.1708-

8208.2008.00084.x.

481. Tabassum A., Walboomers XF., Wolke JG., Meijer GJ., Jansen JA. Bone particles and the undersized surgical technique. *J Dent Res* 2010;**89**(6):581–6. Doi: 10.1177/0022034510363263 [doi].
482. Trisi P., Todisco M., Consolo U., Travaglini D. High versus low implant insertion torque: a histologic, histomorphometric, and biomechanical study in the sheep mandible. *Int J Oral Maxillofac Implants* 2011;**26**(4):837–49.
483. Coelho PG., Marin C., Teixeira HS., Campos FE., Gomes JB., Guastaldi F., et al. Biomechanical evaluation of undersized drilling on implant biomechanical stability at early implantation times. *J Oral Maxillofac Surg* 2013. Doi: 10.1016/j.joms.2012.10.008.
484. Piattelli A., Artese L., Penitente E., Iaculli F., Degidi M., Mangano C., et al. Osteocyte density in the peri-implant bone of implants retrieved after different time periods (4 weeks to 27 years). *J Biomed Mater Res B, Appl Biomater* 2014;**102**(2):239–43. Doi: 10.1002/jbm.b.33000 [doi].
485. Coelho PG., Bonfante EA., Marin C., Granato R., Giro G., Suzuki M. A human retrieval study of plasma-sprayed hydroxyapatite-coated plateau root form implants after 2 months to 13 years in function. *J Long Term Eff Med Implants* 2010;**20**(4):335–42. Doi: 10.19219f54d152c8,758466d960039a59 [pii].
486. Coelho PG., Marin C., Granato R., Suzuki M. Histomorphologic analysis of 30 plateau root form implants retrieved after 8 to 13 years in function. A human retrieval study. *J Biomed Mater Res B Appl Biomater* 2009;**91**(2):975–9. Doi: 10.1002/jbm.b.31455.
487. Iezzi G., Piattelli A., Mangano C., Shibli JA., Vantaggiato G., Frosecchi M., et al. Peri-implant bone tissues around retrieved human implants after time periods longer than 5 years: A retrospective histologic and histomorphometric evaluation of 8 cases. *Odontology* 2014. Doi: 10.1007/s10266-012-0084-z.
488. Martinez H., Davarpanah M., Missika P., Celletti R., Lazzara R. Optimal implant stabilization in low density bone. *Clin Oral Implants Res* 2001;**12**(5):423–32.
489. Venturelli A. A modified surgical protocol for placing implants in the maxillary tuberosity: clinical results at 36 months after loading with fixed partial dentures. *Int J Oral Maxillofac Implant* 1996.
490. Ostman P-O., Hellman M., Sennerby L. Direct Implant Loading in the Edentulous Maxilla Using a Bone Density–

- Adapted Surgical Protocol and Primary Implant Stability Criteria for Inclusion. *Clin Implant Dent Relat Res* 2006. Doi: 10.1111/j.1708-8208.2005.tb00076.x.
491. Alghamdi H., Anand PS., Anil S. Undersized implant site preparation to enhance primary implant stability in poor bone density: a prospective clinical study. *J Oral Maxillofac Surg* 2011;**69**(12):506. Doi: 10.1016/j.joms.2011.08.007 [doi].
 492. Tabassum A., Meijer GJ., Wolke JGC., Jansen JA. Influence of the surgical technique and surface roughness on the primary stability of an implant in artificial bone with a density equivalent to maxillary bone: A laboratory study. *Clin Oral Implants Res* 2009;**20**(4):327–32. Doi: 10.1111/j.1600-0501.2008.01692.x.
 493. Menezes HHM., Naves MM., Costa HL., Barbosa TP., Ferreira JA., Magalhães D., et al. Effect of Surgical Installation of Dental Implants on Surface Topography and Its Influence on Osteoblast Proliferation. *Int J Dent* 2018;**2018**:4089274. Doi: 10.1155/2018/4089274.
 494. Aldahlawi S., Demeter A., Irinakis T. The effect of implant placement torque on crestal bone remodeling after 1 year of loading. *Clin Cosmet Investig Dent* 2018;**10**:203–9. Doi: 10.2147/CCIDE.S174895.
 495. Tabassum A., Meijer GJ., Wolke JGC., Jansen JA. Influence of surgical technique and surface roughness on the primary stability of an implant in artificial bone with different cortical thickness: A laboratory study. *Clin Oral Implants Res* 2010;**21**(2):213–20. Doi: 10.1111/j.1600-0501.2009.01823.x.
 496. Jimbo R., Tovar N., Anchietà RB., MacHado LS., Marin C., Teixeira HS., et al. The combined effects of undersized drilling and implant macrogeometry on bone healing around dental implants: An experimental study. *Int J Oral Maxillofac Surg* 2014. Doi: 10.1016/j.ijom.2014.03.017.
 497. Rigsby DF., Bidez MW., Misch CE. Bone Response to Mechanical Loads. In: Misch CE, editor. *Contemporary implant dentistry*. 2nd ed. St. Louis: Mosby; 1998. p. 317–28.
 498. Koistinen AP., Korhonen H., Kiviranta I., Kröger H., Lappalainen R. Analysis of plastic deformation in cortical bone after insertion of coated and non-coated self-tapping orthopaedic screws. *Proc Inst Mech Eng H* 2011;**225**(7):629–39. Doi: 10.1177/0954411911406471.
 499. Warreth A., Polyzois I., Lee CT., Claffey N. Generation of microdamage around endosseous implants. *Clin Oral Implants Res* 2009;**20**(12):1300–6. Doi: 10.1111/j.1600-0501.2009.01808.x [doi].

500. Campos., Gomes JB., Marin C., Teixeira HS., Suzuki M., Witek L., et al. Effect of drilling dimension on implant placement torque and early osseointegration stages: an experimental study in dogs. *J Oral Maxillofac Surg* 2012;**70**(1):43.
501. Jung U-W., Kim S., Kim Y-H., Cha J-K., Lee I-S., Choi S-H. Osseointegration of dental implants installed without mechanical engagement: a histometric analysis in dogs. *Clin Oral Implants Res* 2012;**23**(11):1297–301. Doi: 10.1111/j.1600-0501.2011.02306.x.
502. Duyck J., Roesems R., Cardoso M V., Ogawa T., De Villa Camargos G., Vandamme K. Effect of insertion torque on titanium implant osseointegration: an animal experimental study. *Clin Oral Implants Res* 2015;**26**(2):191–6. Doi: 10.1111/clr.12316.
503. Toia M., Stocchero M., Cecchinato F., Corra E., Jimbo R., Cecchinato D. Clinical Considerations of Adapted Drilling Protocol by Bone Quality Perception. *Int J Oral Maxillofac Implants* 2017. Doi: 10.11607/jomi.5881.
504. Sugiura T., Yamamoto K., Kawakami M., Horita S., Murakami K., Kirita T. Influence of bone parameters on peri-implant bone strain distribution in the posterior mandible. *Med Oral Patol Oral Cir Bucal* 2015. Doi: 10.4317/medoral.19878.
505. Mercer C., He MY., Wang R., Evans AG. Mechanisms governing the inelastic deformation of cortical bone and application to trabecular bone. *Acta Biomater* 2006;**2**(1):59–68. Doi: 10.1016/j.actbio.2005.08.004.
506. Tang T., Ebacher V., Crompton P., Guy P., McKay H., Wang R. Shear deformation and fracture of human cortical bone. *Bone* 2015;**71**:25–35. Doi: 10.1016/j.bone.2014.10.001.
507. Zimmermann EA., Gludovatz B., Schaible E., Busse B., Ritchie RO. Fracture resistance of human cortical bone across multiple length-scales at physiological strain rates. *Biomaterials* 2014. Doi: 10.1016/j.biomaterials.2014.03.066.
508. Shah KN., Racine J., Jones LC., Aaron RK. Pathophysiology and risk factors for osteonecrosis. *Curr Rev Musculoskelet Med* 2015;**8**(3):201–9. Doi: 10.1007/s12178-015-9277-8.
509. Kennedy Damien M. | Majeska Robert J. | Sun Hui B. | Schaffler Mitchell B OD | Laudie. Osteocyte apoptosis is required for production of osteoclastogenic signals following bone fatigue in vivo. *Bone* 2014;**64**:132–7. Doi: 10.1016/j.bone.2014.03.049.
510. Verborgt O., Gibson GJ., Schaffler MB. Loss of osteocyte

- integrity in association with microdamage and bone remodeling after fatigue in vivo. *J Bone Miner Res Off J Am Soc Bone Miner Res* 2000;**15**(1):60–7. Doi: 10.1359/jbmr.2000.15.1.60.
511. Stocchero M., Toia M., Jinno Y., Cecchinato F., Becktor JP., Naito Y., et al. Influence of different drilling preparation on cortical bone: A biomechanical, histological, and micro-CT study on sheep. *Clin Oral Implants Res* 2018;**29**(7):707–15. Doi: 10.1111/clr.13262.
 512. Pantani F., Botticelli D., Garcia Idelmo Rangel J., Salata LA., Borges GJ., Lang NP. Influence of lateral pressure to the implant bed on osseointegration: an experimental study in dogs. *Clin Oral Implants Res* 2010;**21**(11):1264. Doi: 10.1111/j.1600-0501.2010.01941.x.
 513. Tabassum A., Meijer GJ., Walboomers XF., Jansen JA. Evaluation of primary and secondary stability of titanium implants using different surgical techniques. *Clin Oral Implants Res* 2014;**25**(4):487–92. Doi: 10.1111/clr.12180.
 514. Al-Marshood MM., Junker R., Al-Rasheed A., Al Farraj Aldosari A., Jansen JA., Anil S. Study of the osseointegration of dental implants placed with an adapted surgical technique. *Clin Oral Implants Res* 2011;**22**(7):753–9. Doi: 10.1111/j.1600-0501.2010.02055.x [doi].
 515. Stavropoulos A., Cochran D., Obrecht M., Pippenger BE., Dard M. Effect of Osteotomy Preparation on Osseointegration of Immediately Loaded, Tapered Dental Implants. *Adv Dent Res* 2016. Doi: 10.1177/0022034515624446.
 516. Cohen O., Ormianer Z., Tal H., Rothamel D., Weinreb M., Moses O. Differences in crestal bone-to-implant contact following an under-drilling compared to an over-drilling protocol. A study in the rabbit tibia. *Clin Oral Investig* 2016;**20**(9):2475–80. Doi: 10.1007/s00784-016-1765-8.
 517. Sugiura T., Yamamoto K., Horita S., Murakami K., Tsutsumi S., Kirita T. The effects of bone density and crestal cortical bone thickness on micromotion and peri-implant bone strain distribution in an immediately loaded implant: a nonlinear finite element analysis. *J Periodontal Implant Sci* 2016;**46**(3):152–65. Doi: 10.5051/jpis.2016.46.3.152.
 518. Bashutski JD., D'Silva NJ., Wang HL. Implant compression necrosis: current understanding and case report. *J Periodontol* 2009;**80**(4):700–4. Doi: 10.1902/jop.2009.080581 [doi].
 519. Abrahamsson I., Linder E., Lang NP. Implant stability in relation to osseointegration: an experimental study in the Labrador dog. *Clin Oral Implants Res* 2009;**20**(3):313–8.

520. Marin C., Granato R., Suzuki M., Gil JN., Janal MN., Coelho PG. Histomorphologic and histomorphometric evaluation of various endosseous implant healing chamber configurations at early implantation times: A study in dogs. *Clin Oral Implants Res* 2010;**21**(6):577–83. Doi: 10.1111/j.1600-0501.2009.01853.x.
521. Norton M. The Influence of Low Insertion Torque on Primary Stability, Implant Survival, and Maintenance of Marginal Bone Levels: A Closed-Cohort Prospective Study. *Int J Oral Maxillofac Implants* 2017;**32**(4):849–57. Doi: 10.11607/jomi.5889.
522. Bonfante EA., Granato R., Marin C., Suzuki M., Oliveira SR., Giro G., et al. Early bone healing and biomechanical fixation of dual acid-etched and as-machined implants with healing chambers: an experimental study in dogs 2011;**26**(1):75–82.
523. Kim M-S., Jung U-W., Kim S., Lee J-S., Lee I-S., Choi S-H. Bone apposition on implants coated with calcium phosphate by ion beam assisted deposition in oversized drilled sockets: a histologic and histometric analysis in dogs. *J Periodontal Implant Sci* 2013;**43**(1):18–23. Doi: 10.5051/jpis.2013.43.1.18.
524. Insua A., Monje A., Wang HL., Miron RJ. Basis of bone metabolism around dental implants during osseointegration and peri-implant bone loss. *J Biomed Mater Res - Part A* 2017. Doi: 10.1002/jbm.a.36060.
525. Berglundh T., Stavropoulos A. Preclinical in vivo research in implant dentistry. Consensus of the eighth European workshop on periodontology. *J Clin Periodontol* 2012;**39** Suppl 12:1–5. Doi: 10.1111/j.1600-051X.2011.01827.x [doi].
526. Brinkmann J., Hefti T., Schlottig F., Spencer ND., Hall H. Response of osteoclasts to titanium surfaces with increasing surface roughness: an in vitro study. *Biointerphases* 2012;**7**(1–4):34. Doi: 10.1007/s13758-012-0034-x.
527. Hsu H., Lacey DL., Dunstan CR., Solovyev I., Colombero A., Timms E., et al. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. *Proc Natl Acad Sci U S A* 1999;**96**(7):3540–5.
528. Collin-Osdoby P., Osdoby P. RANKL-mediated osteoclast formation from murine RAW 264.7 cells. *Methods Mol Biol* 2012;**816**:187–202. Doi: 10.1007/978-1-61779-415-5_13 [doi].
529. Kong L., Smith W., Hao D. Overview of RAW264.7 for osteoclastogenesis study: Phenotype and stimuli. *J Cell Mol*

- Med* 2019. Doi: 10.1111/jcmm.14277.
530. Alberts B., Johnson A., Lewis J., Raff M., Roberts K., Walter P. Cell Chemistry and Biosynthesis. *Molecular biology of the cell*. 6th ed. New York: Garland Science; 2014.
 531. Griffiths AJF. *Modern genetic analysis*. W.H. Freeman; 1999.
 532. Alberts B., Johnson A., Lewis J., Raff M., Roberts K., Walter P. DNA, Chromosomes and Genomes. *Molecular Biology of the Cell*. 6th ed. New York: Garland Science; 2014.
 533. De Sousa Abreu R., Penalva LO., Marcotte EM., Vogel C. Global signatures of protein and mRNA expression levels. *Mol Biosyst* 2009. Doi: 10.1039/b908315d.
 534. Maier T., Güell M., Serrano L. Correlation of mRNA and protein in complex biological samples. *FEBS Lett* 2009. Doi: 10.1016/j.febslet.2009.10.036.
 535. Delidow BC., Peluso JJ., White BA. Quantitative measurement of mRNAs by polymerase chain reaction. *Gene Anal Tech* n.d.;**6**(6):120–4.
 536. Bachman J. Reverse-Transcription PCR (RT-PCR). *Methods Enzymol* 2013;**530**:67–74. Doi: 10.1016/B978-0-12-420037-1.00002-6.
 537. Mullis KB., Faloona FA. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol* 1987;**155**:335–50.
 538. Nolan T., Bustin SA. *PCR Technology: Current Innovations*. 3rd ed. CRC Press; 2016.
 539. Higuchi R., Fockler C., Dollinger G., Watson R. Kinetic PCR analysis: Real-time monitoring of DNA amplification reactions. *Nat Biotechnol* 1993. Doi: 10.1038/nbt0993-1026.
 540. Chien A., Edgar DB., Trela JM. Deoxyribonucleic acid polymerase from the extreme thermophile *Thermus aquaticus*. *J Bacteriol* 1976;**127**(3):1550–7.
 541. Holland PM., Abramson RD., Watson R., Gelfand DH. Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proc Natl Acad Sci U S A* 1991;**88**(16):7276–80.
 542. Bass C., Nikou D., Vontas J., Donnelly MJ., Williamson MS., Field LM. The Vector Population Monitoring Tool (VPMT): High-Throughput DNA-Based Diagnostics for the Monitoring of Mosquito Vector Populations. *Malar Res Treat* 2010;**2010**:190434. Doi: 10.4061/2010/190434.
 543. Kutyavin I V., Afonina IA., Mills A., Gorn V V., Lukhtanov EA., Belousov ES., et al. 3'-minor groove binder-DNA probes increase sequence specificity at PCR extension temperatures.

Nucleic Acids Res 2000.

544. Tajadini M., Panjehpour M., Javanmard S. Comparison of SYBR Green and TaqMan methods in quantitative real-time polymerase chain reaction analysis of four adenosine receptor subtypes. *Adv Biomed Res* 2014. Doi: 10.4103/2277-9175.127998.
545. Bustin SA., Benes V., Garson JA., Hellemans J., Huggett J., Kubista M., et al. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* 2009. Doi: 10.1373/clinchem.2008.112797.
546. Livak KJ., Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods* 2001. Doi: 10.1006/meth.2001.1262.
547. Stephens AS., Stephens SR., Morrison NA. Internal control genes for quantitative RT-PCR expression analysis in mouse osteoblasts, osteoclasts and macrophages. *BMC Res Notes* 2011;4:410. Doi: 10.1186/1756-0500-4-410.
548. Vandesompele J., Preter D., Pattyn F., Poppe B., Van Roy N., De Paepe A., et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002. Doi: 10.1186/gb-2002-3-7-research0034.
549. Lung CYK., Matinlinna JP. Surface Pretreatment Methods and Silanization. In: Matinlinna JP, editor. *Handbook of Oral Biomaterials*. Singapore: Pan Stanford Publishing; 2014. p. 359–97.
550. W. Burry R. Immunocytochemistry. *Immunocytochemistry*. New York: Springer; 2010. p. 7–16.
551. Lakowicz JR. Introduction to Fluorescence. *Principles of Fluorescence Spectroscopy*. 3rd ed. Springer Science & Business Media; 2006. p. 1–26.
552. Guy Cox. Optical Imaging Techniques in Cell Biology. 1st ed. CRC Press; 2006. p. 63–82.
553. Schachtner H., Calaminus SDJ., Thomas SG., Machesky LM. Podosomes in adhesion, migration, mechanosensing and matrix remodeling. *Cytoskeleton (Hoboken)* 2013;70(10):572–89. Doi: 10.1002/cm.21119.
554. Green S., Higgins JP. *Systematic reviews of interventions*. 2008.
555. Vesterinen HM., Sena ES., Egan KJ., Hirst TC., Churolov L., Currie GL., et al. Meta-analysis of data from animal studies: a practical guide. *J Neurosci Methods* 2014;221:92–102. Doi: 10.1016/j.jneumeth.2013.09.010 [doi].

556. Schardt C., Adams MB., Owens T., Keitz S., Fontelo P. Utilization of the PICO framework to improve searching PubMed for clinical questions. *BMC Med Inform Decis Mak* 2007;**7**:16. Doi: 10.1186/1472-6947-7-16.
557. Cipriani A., Geddes J. Comparison of systematic and narrative reviews: The example of the atypical antipsychotics. *Epidemiol Psychiatr Soc* 2003. Doi: 10.1017/S1121189X00002918.
558. Higgins JP., Altman DG. Assessing Risk of Bias in Included Studies. *Cochrane Handbook for Systematic Reviews of Interventions: Cochrane Book Series*. 2008.
559. Moher D., Shamseer L., Clarke M., Ghersi D., Liberati A., Petticrew M., et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Rev Esp Nutr Humana y Diet* 2016. Doi: 10.1186/2046-4053-4-1.
560. Lefebvre C., Manheimer E., Glanville J. Searching for Studies. *Cochrane Handbook for Systematic Reviews of Interventions: Cochrane Book Series*. 2008.
561. Higgins JP., Deeks JJ. Selecting Studies and Collecting Data. *Cochrane Handbook for Systematic Reviews of Interventions: Cochrane Book Series*. 2008.
562. Schünemann HJ., Oxman AD., Higgins JP., Vist GE., Glasziou PP., Guyatt GH. Presenting Results and “Summary of Findings” Tables. *Cochrane Handbook for Systematic Reviews of Interventions: Cochrane Book Series*. 2008.
563. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Medica* 2012;**22**(3):276–82.
564. Hooijmans CR., Rovers MM., de Vries RB., Leenaars M., Ritskes-Hoitinga M., Langendam MW. SYRCLE’s risk of bias tool for animal studies. *BMC Med Res Methodol* 2014;**14**:43. Doi: 10.1186/1471-2288-14-43 [doi].
565. Donath K., Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Säge-Schliff (sawing and grinding) technique. *J Oral Pathol* 1982;**11**(4):318–26.
566. Donath K. Preparation of histologic sections by the cutting-grinding technique for hard tissue and other material not suitable to be sectioned by routine methods. *Nord Exakt-Kulzer-Publication* 1993:1–16.
567. Buser D., Nydegger T., Hirt HP., Cochran DL., Nolte LP. Removal torque values of titanium implants in the maxilla of miniature pigs. *Int J Oral Maxillofac Implants* 1998;**13**(5):611–9.
568. Kim S-H., Lee S-J., Cho I-S., Kim S-K., Kim T-W. Rotational

- resistance of surface-treated mini-implants. *Angle Orthod* 2009;**79**(5):899–907.
569. Riffenburgh RH. *Statistics in Medicine*. 3rd ed. San Diego: Academic Press; 2012.
 570. Askie L., Offringa M. Systematic reviews and meta-analysis. *Semin Fetal Neonatal Med* 2015. Doi: 10.1016/j.siny.2015.10.002.
 571. Deeks JJ., Higgins JPT., Altman DG. Analysing Data and Undertaking Meta-Analyses. In: Higgins JPT, and Green S, editors. *Cochrane Handbook for Systematic Reviews of Interventions: Cochrane Book Series*. John Wiley & Sons, Ltd; 2008. p. 243–96.
 572. Hanks CT., Wataha JC., Sun Z. In vitro models of biocompatibility: A review. *Dent Mater* 1996. Doi: 10.1016/S0109-5641(96)80020-0.
 573. Underhill GH., Peter G., Chen CS., Bhatia SN. Bioengineering Methods for Analysis of Cells In Vitro. *Annu Rev Cell Dev Biol* 2012. Doi: 10.1146/annurev-cellbio-101011-155709.
 574. Freshney RI. *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications*. 7th ed. Wiley-Blackwell; 2016.
 575. Gartland A., Rumney RMH., Dillon JP., Gallagher JA. Isolation and culture of human osteoblasts. *Methods Mol Biol* 2012. Doi: 10.1007/978-1-61779-367-7_22.
 576. Dillon JP., Waring-Green VJ., Taylor AM., Wilson PJM., Birch M., Gartland A., et al. Primary human osteoblast cultures. *Methods Mol Biol* 2012. Doi: 10.1007/978-1-61779-415-5_1.
 577. Clover J., Gowen M. Are MG-63 and HOS TE85 human osteosarcoma cell lines representative models of the osteoblastic phenotype? *Bone* 1994. Doi: 10.1016/8756-3282(94)90305-0.
 578. Czekanska EM., Stoddart MJ., Ralphs JR., Richards RG., Hayes JS. A phenotypic comparison of osteoblast cell lines versus human primary osteoblasts for biomaterials testing. *J Biomed Mater Res - Part A* 2014. Doi: 10.1002/jbm.a.34937.
 579. Dar HY., Azam Z., Anupam R., Mondal RK., Srivastava RK. Osteoimmunology: The Nexus between bone and immune system. *Front Biosci (Landmark Ed)* 2018;**23**:464–92.
 580. Franz S., Rammelt S., Scharnweber D., Simon JC. Immune responses to implants - A review of the implications for the design of immunomodulatory biomaterials. *Biomaterials* 2011. Doi: 10.1016/j.biomaterials.2011.05.078.
 581. Stavropoulos A., Sculean A., Bosshardt DD., Buser D., Klinge B. Pre-clinical in vivo models for the screening of bone

- biomaterials for oral/craniofacial indications: focus on small-animal models. *Periodontol 2000* 2015;**68**(1):55–65. Doi: 10.1111/prd.12065 [doi].
582. World Medical Association. World Medical Association Declaration of Helsinki. *JAMA* 2013;**310**(20):2191. Doi: 10.1001/jama.2013.281053.
 583. Muschler GF., Raut VP., Patterson TE., Wenke JC., Hollinger JO. The design and use of animal models for translational research in bone tissue engineering and regenerative medicine. *Tissue Eng Part B Rev* 2010;**16**(1):123–45. Doi: 10.1089/ten.TEB.2009.0658.
 584. Aerssens J., Boonen S., Lowet G., Dequeker J. Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. *Endocrinology* 1998;**139**(2):663–70. Doi: 10.1210/endo.139.2.5751.
 585. Donos N., Dereka X., Mardas N. Experimental models for guided bone regeneration in healthy and medically compromised conditions. *Periodontol 2000* 2015. Doi: 10.1111/prd.12077.
 586. Pearce AI., Richards RG., Milz S., Schneider E., Pearce SG. Animal models for implant biomaterial research in bone: a review. *Eur Cell Mater* 2007;**13**(1):1–10.
 587. Vignoletti F., Abrahamsson I. Quality of reporting of experimental research in implant dentistry. Critical aspects in design, outcome assessment and model validation. *J Clin Periodontol* 2012;**39** Suppl 12:6–27. Doi: 10.1111/j.1600-051X.2011.01830.x [doi].
 588. Stadlinger B., Pourmand P., Locher MC., Schulz MC. Systematic review of animal models for the study of implant integration, assessing the influence of material, surface and design. *J Clin Periodontol* 2012;**39** Suppl 12:28–36. Doi: 10.1111/j.1600-051X.2011.01835.x [doi].
 589. Allen MJ., Hankenson KD., Goodrich L., Boivin GP., von Rechenberg B. Ethical use of animal models in musculoskeletal research. *J Orthop Res* 2017. Doi: 10.1002/jor.23485.
 590. Lesaffre E., Philstrom B., Needleman I., Worthington H. The design and analysis of split-mouth studies: What statisticians and clinicians should know. *Statistics in Medicine*. 2009.
 591. Kortegaard HE., Eriksen T., Baelum V. Screening for periodontal disease in research dogs - a methodology study. *Acta Vet Scand* 2014;**56**(1):77. Doi: 10.1186/s13028-014-0077-8.
 592. Coelho PG., Freire JN., Granato R., Marin C., Bonfante EA., Gil JN., et al. Bone mineral apposition rates at early

- implantation times around differently prepared titanium surfaces: a study in beagle dogs. *Int J Oral Maxillofac Implants* 2011;**26**(1):63–9.
593. Corte GM., Plendl J., Hünigen H., Richardson KC., Gemeinhardt O., Niehues SM. Refining experimental dental implant testing in the Göttingen Minipig using 3D computed tomography - A morphometric study of the mandibular canal. *PLoS One* 2017. Doi: 10.1371/journal.pone.0184889.
 594. Simons W-F., De Smit M., Duyck J., Coucke W., Quirynen M. The proportion of cancellous bone as predictive factor for early marginal bone loss around implants in the posterior part of the mandible. *Clin Oral Implants Res* 2015;**26**(9):1051–9. Doi: 10.1111/clr.12398.
 595. Qahash M., Susin C., Polimeni G., Hall J., Wikesjö UME. Bone healing dynamics at buccal peri-implant sites. *Clin Oral Implants Res* 2008;**19**(2):166–72. Doi: 10.1111/j.1600-0501.2007.01428.x.
 596. Bornstein MM., Valderrama P., Jones AA., Wilson TG., Seibl R., Cochran DL. Bone apposition around two different sandblasted and acid-etched titanium implant surfaces: a histomorphometric study in canine mandibles. *Clin Oral Implants Res* 2008;**19**(3):233–41. Doi: 10.1111/j.1600-0501.2007.01473.x [doi].
 597. Rossi F., Lang NP., De Santis E., Morelli F., Favero G., Botticelli D. Bone-healing pattern at the surface of titanium implants: An experimental study in the dog. *Clin Oral Implants Res* 2014. Doi: 10.1111/clr.12097.
 598. Mainetti T., Lang NP., Bengazi F., Sbricoli L., Soto Cantero L., Botticelli D. Immediate loading of implants installed in a healed alveolar bony ridge or immediately after tooth extraction: An experimental study in dogs. *Clin Oral Implants Res* 2015. Doi: 10.1111/clr.12389.
 599. Lang NP., Salvi GE., Huynh-Ba G., Ivanovski S., Donos N., Bosshardt DD. Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. *Clin Oral Implants Res* 2011;**22**(4):349–56. Doi: 10.1111/j.1600-0501.2011.02172.x [doi].
 600. Sartoretto SC., Alves ATNN., Zarranz L., Jorge MZ., Granjeiro JM., Calasans-Maia MD. Hydrophilic surface of Ti6Al4V-ELI alloy improves the early bone apposition of sheep tibia. *Clin Oral Implants Res* 2017. Doi: 10.1111/clr.12894.
 601. Sartoretto SC., Calasans-Maia J de A., da Costa YO., Louro RS., Granjeiro JÉ., Calasans-Maia MD. Accelerated healing period with hydrophilic implant placed in sheep Tibia. *Braz*

- Dent J* 2017. Doi: 10.1590/0103-6440201601559.
602. Wennerberg A., Albrektsson T. Effects of titanium surface topography on bone integration: a systematic review. *Clin Oral Implants Res* 2009;**20 Suppl 4**:172–84. Doi: 10.1111/j.1600-0501.2009.01775.x [doi].
 603. Shanbhag S., Shanbhag V., Stavropoulos A. Genomic analyses of early peri-implant bone healing in humans: a systematic review. *Int J Implant Dent* 2015;**1**(1):5. Doi: 10.1186/s40729-015-0006-2.
 604. Marin C., Granato R., Suzuki M., Gil JN., Piattelli A., Coelho PG. Removal Torque and Histomorphometric Evaluation of Bioceramic Grit-Blasted/Acid-Etched and Dual Acid-Etched Implant Surfaces: An Experimental Study in Dogs. *J Periodontol* 2008;**79**(10):1942–9. Doi: 10.1902/jop.2008.080106.
 605. Georgess D., Machuca-Gayet I., Blangy A., Jurdic P. Podosome organization drives osteoclast-mediated bone resorption. *Cell Adhes Migr* 2014. Doi: 10.4161/cam.27840.
 606. Geblinger D., Addadi L., Geiger B. Nano-topography sensing by osteoclasts. *J Cell Sci* 2010. Doi: 10.1242/jcs.060954.
 607. Geblinger D., Zink C., Spencer ND., Addadi L., Geiger B. Effects of surface microtopography on the assembly of the osteoclast resorption apparatus. *J R Soc Interface* 2012. Doi: 10.1098/rsif.2011.0659.
 608. Solberg LB., Brorson SH., Stordalen GA., Bækkevold ES., Andersson G., Reinholt FP. Increased tartrate-resistant acid phosphatase expression in osteoblasts and osteocytes in experimental osteoporosis in rats. *Calcif Tissue Int* 2014. Doi: 10.1007/s00223-013-9834-3.
 609. Saltel F., Bard F., Destaing O., Eichert D., Jurdic P. Apatite-mediated Actin Dynamics in Resorbing Osteoclasts. *Mol Biol Cell* 2004. Doi: 10.1091/mbc.e04-06-0522.
 610. Barth KA., Waterfield JD., Brunette DM. The effect of surface roughness on RAW 264.7 macrophage phenotype. *J Biomed Mater Res A* 2013;**101**(9):2679–88. Doi: 10.1002/jbm.a.34562 [doi].
 611. Scheller J., Chalaris A., Schmidt-Arras D., Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta - Mol Cell Res* 2011;**1813**(5):878–88. Doi: 10.1016/j.bbamcr.2011.01.034.
 612. Yurttutan ME., Kestane R., Keskin A., Dereci Ö. Biomechanical evaluation of oversized drilling on implant stability-an experimental study in sheep. *J Pak Med Assoc* 2016. Doi: 10.1002/prot.22859.

613. Slaets E., Carmeliet G., Naert I., Duyck J. Early cellular responses in cortical bone healing around unloaded titanium implants: an animal study. *J Periodontol* 2006;**77**(6):1015–24. Doi: 10.1902/jop.2006.050196.
614. Hudieb M., Wakabayashi N., Suzuki T., Kasugai S. Morphologic classification and stress analysis of the mandibular bone in the premolar region for implant placement. *Int J Oral Maxillofac Implants* 2010;**25**(3):482–90.
615. Sugiura T., Horiuchi K., Sugimura M., Tsutsumi S. Evaluation of threshold stress for bone resorption around screws based on in vivo strain measurement of miniplate. *J Musculoskelet Neuronal Interact* 2000;**1**(2):165–70.
616. Berardini M., Trisi P., Sinjari B., Rutjes AWS., Caputi S. The effects of high insertion torque versus low insertion torque on marginal bone resorption and implant failure rates: A systematic review with meta-analyses. *Implant Dent* 2016. Doi: 10.1097/ID.0000000000000422.
617. Duyck J., Rønold HJ., Van Oosterwyck H., Naert I., Vander Sloten J., Ellingsen JE. The influence of static and dynamic loading on marginal bone reactions around osseointegrated implants: an animal experimental study. *Clin Oral Implants Res* 2001;**12**(3):207–18.
618. Rea M., Botticelli D., Ricci S., Soldini C., González GG., Lang NP. Influence of immediate loading on healing of implants installed with different insertion torques – an experimental study in dogs. *Clin Oral Implants Res* 2015;**26**(1):90–5. Doi: 10.1111/clr.12305.
619. Marin C., Bonfante EA., Jeong R., Granato R., Giro G., Suzuki M., et al. Histologic and biomechanical evaluation of 2 resorbable-blasting media implant surfaces at early implantation times. *J Oral Implantol* 2013;**39**(4):445–53. Doi: 10.1563/AAID-JOI-D-10-00156 [doi].
620. Yamanishi Y., Yamaguchi S., Imazato S., Nakano T., Yatani H. Effects of the implant design on peri-implant bone stress and abutment micromovement: three-dimensional finite element analysis of original computer-aided design models. *J Periodontol* 2014;**85**(9):333. Doi: 10.1902/jop.2014.140107 [doi].
621. Coelho PG., Jimbo R., Tovar N., Bonfante EA. Osseointegration: hierarchical designing encompassing the micrometer, micrometer, and nanometer length scales. *Dent Mater Off Publ Acad Dent Mater* 2015;**31**(1):37–52. Doi: 10.1016/j.dental.2014.10.007.
622. de Jesus RNR., Carrilho E., Antunes P V., Ramalho A., Moura

- CCG., Stavropoulos A., et al. Interfacial biomechanical properties of a dual acid-etched versus a chemically modified hydrophilic dual acid-etched implant surface: an experimental study in Beagles. *Int J Implant Dent* 2018;**4**(1):28. Doi: 10.1186/s40729-018-0139-1.
623. de Jesus RNR., Stavropoulos A., Oliveira MTF., Soares PBF., Moura CCG., Zanetta-Barbosa D. Histomorphometric evaluation of a dual acid-etched vs. a chemically modified hydrophilic dual acid-etched implant surface. An experimental study in dogs. *Clin Oral Implants Res* 2017;**28**(5):551–7. Doi: 10.1111/clr.12833.

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Histomorphometric evaluation of a dual acid-etched vs. a chemically modified hydrophilic dual acid-etched implant surface. An experimental study in dogs

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Key words: bone-to-implant contact, dogs, implant surfaces, oral implants, osseointegration

Abstract

Objective: The aim of this preclinical *in vivo* study was to compare histologically and histomorphometrically osseointegration of dual acid-etched vs. hydrophilic implants.

Material and methods: Two pairs of implants (Neodent, Curitiba, Brazil), with same macrogeometry but different surface technology (i) dual acid-etched surface (SAE) treatment with hydrochloric and sulfuric acid followed by microwave treatment and insertion in isotonic saline solution to increase hydrophilicity (SAE-HD) (test, $n = 12$); (ii) dual SAE (control, $n = 12$) were installed bilaterally in the proximal tibia of six beagle dogs. Histologic and histomorphometric evaluation was performed after 2 and 4 weeks *in vivo*, on non-decalcified sections. Percentages of bone-to-implant contact (BIC) and bone density (BD) were estimated and tested for significant differences with the Wilcoxon signed-rank test for paired samples ($P < 0.05$).

Results: In general, new bone formation along and in contact with the implant surface could be observed irrespective of the experimental group and observation period. Most of the bone was woven but small quantities of lamellar bone, mainly in close proximity to the cortex could also be observed. BIC at 2 weeks was 19.57 ± 13.57 and 20.33 ± 7.99 ($P = 0.75$), and at 4 weeks was 42.80 ± 14.48 and 40.25 ± 9.45 ($P = 0.65$) for SAE-HD and SAE implants respectively. BD at 2 weeks was 24.85 ± 16.31 and 25.66 ± 8.59 ($P = 0.35$) and at 4 weeks 44.13 ± 6.46 and 40.13 ± 6.46 ($P = 0.25$) for SAE-HD and SAE implants respectively.

Conclusion: Bone-to-implant contact and BD increased with time in both SAE-HD and SAE implants. No significant differences were observed between the two different implant surfaces for any of the evaluated parameters and at any observation time-point.

Oral implant rehabilitation of edentulous patients has become a predictable routine treatment, with high survival rates (90–95%) being reported for the major dental implant systems after 5–10 years in function (Ferrigno et al. 2002; Albrektsson & Wennerberg 2004; Balshi et al. 2005; Meijer et al. 2009; Barawal et al. 2012; Jimbo & Albrektsson 2015). The high efficacy and clinical success of contemporary implants is based, among other parameters, on advances in implant surface technology; specifically, modification of the titanium implant surface topography resulting into a moderately rough surface (Ra/

values between 1 and 2 μm) has been shown to enhance osseointegration (i.e. achieve faster and/or larger amounts of mineralized bone-to-implant contact [BIC]) compared to the minimally rough surfaced machined implants (Albrektsson & Wennerberg 2004; Wennerberg & Albrektsson 2009, 2010; Elias & Meirelles 2010).

In this context, increased implant surface roughness can be achieved by a variety of manufacturing methods, where the most common are: sandblasting, acid-etching, anodic oxidation, and laser modification, or combinations thereof (Dohan Ehrenfest et al.

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2010]. Preclinical *in vivo* studies have shown that a moderately rough sandblasted, large grid, acid-etched implant surface modification (SLA) results in a better BIC and higher removal torque values at early stages of bone healing (up to 3 months) compared with minimally rough machined and titanium plasma-sprayed surfaces (Buser et al. 1999; Al-Nawas et al. 2007). The potential of achieving greater and better quality of bone anchorage over time with this surface treatment allows immediate or early functional loading of the implants (Cochran et al. 1998; Li et al. 2002; Quinlan et al. 2005; Chambrone et al. 2015; Kim et al. 2015), and excellent results have been reported at sites of relatively poor bone quality (e.g. in the posterior maxilla) (Khang et al. 2001; Al-Nawas et al. 2007). Furthermore, high levels of clinical success with minimal marginal bone loss on the long-term were recently reported for implants with this type of surface technology (Derks et al. 2015).

Another approach to enhance osseointegration, in addition to modifying implant surface topography, is to change implant surface chemistry, for example, by achieving high surface energy and hydrophilicity. A SLA surface, where the acid-etching procedure is performed under N₂ protection and the surface then directly stored in an isotonic saline solution (SLA-mod), has been shown to possess higher osteogenic surface properties than the ordinary sandblasted acid-etched surface (SAE) (Rupp et al. 2006; Schwarz et al. 2009). Specifically, this surface modification ensures rapid coverage of the implant surface with blood and enhances clot formation and adhesion of osteogenic cells (Buser et al. 2004; Schwarz et al. 2007; Mamlis & Silvestros 2011). Furthermore, it has recently been reported from *in vitro* analyses that implant surface hydrophilicity enhances expression of various angiogenesis factors, i.e. vascular endothelial growth factor-A (VEGF-A), epidermal growth factor (EGF) (An et al. 2010; Raines et al. 2010) and osteogenic differentiation of human osteoblast-like cells (Masaki et al. 2005; Zhao et al. 2005; Mamlis & Silvestros 2011). Preclinical *in vivo* studies have shown that implants with such chemical modification of their surface achieve – in a shorter period of time – larger amounts of mineralized BIC compared to non-modified implants with otherwise similar surface topography (Buser et al. 2004; Schwarz et al. 2008; Vasak et al. 2013; Sartoretto et al. 2015). In the clinic, implants with this type of surface technology show high implant stability quotient values during early healing

time-points (Wennerberg et al. 2011), where otherwise a drop in mineralized BIC (experienced as drop in implant stability values) occurs due to remodeling/resorption of bone in direct contact with the implant after insertion (Gomes et al. 2013); further, marginal peri-implant bone loss <1 mm, on average, over the first 3 years are reported for such implants (Wennerberg et al. 2011).

Despite the excellent performance of most major implant systems, much research on new implant designs and/or surface modifications for enhancing BIC is propagated by established or new implant manufactures. Evaluation of the effect of modifications in implant design and surface topography and/or chemistry composition on the bone response in *in vivo* preclinical models using small- and large animal platforms is mandatory prior to clinical testing, also for regulatory reasons (Berglundh & Stavropoulos 2012). Thus, the objective of this preclinical *in vivo* study was to compare mineralized BIC and relative peri-implant bone density (BD) at implants with a sandblasted, dual SAE, with or without an additional chemical modification (SAE-HD and SAE respectively). It was hypothesized that SE-HD implants would achieve higher BIC compared with SAE implants, at early stages of healing.

Material and methods

The present preclinical *in vivo* study was approved by the ethics committee of the Federal University of Uberlândia (approval number 098/10), and was conducted between November 2012 and January 2013. The present study is reported according to the ARRIVE guidelines, in regard with relevant items (Berglundh & Stavropoulos 2012).

Experimental units

Twenty-four commercially pure titanium implants with a moderately rough surface, produced by means of sandblasting and dual acid-etching with hydrochloric and sulfuric acid were used in the present study. This specific implant surface exhibits the following 3D surface roughness parameters; Sa: 1.26–1.44 µm, Sz: 14.57–16.20 µm, Sdr: 0.01–0.02, Sda: 0, Sdh: 0, and Sds: 643.3–658.7 1/(mm²). Implants in the control group were only sandblasted and dual acid-etched (SAE; *n* = 12). In the test group, after sandblasting and dual acid-etching, implants received proprietary technology treatment, including microwave treatment and insertion in isotonic saline solution resulting in significantly

increased hydrophilicity (SAE-HD; *n* = 12). Neodent® (Curitiba, PR, Brazil) supplied all implants.

Surgical procedures

Six male beagle dogs (~1.5 years of age) were used in the present study. The animals were kept in the animal care facility of Federal University of Uberlândia, received hard-pellet food twice per day, and had ad libitum access to water. After acclimatization in the facilities for 1 week, operations were performed as follows. Sedation was achieved with an intramuscular injection of atropine sulfate (0.044 mg/kg) (Faramagrica S.A., São Paulo, SP, Brazil) and xilazine chlorate (8 mg/kg) (Virbac do Brasil Indústria e Comércio Ltda., São Paulo, SP, Brazil). Then, general anesthesia was induced with 15 mg/kg ketamine chlorate (Faramagrica S.A.). Intra- and peri-operative infection control was achieved with a prophylactic injection of Pentabiotic (30,000 UI/kg; i.m.) (Pentabiotico Veterinário® 1,200,000 UI. Fort Dodge Saúde Animal Ltda. Campinas, SP, Brazil).

Each proximal tibia was initially shaved with a razor blade followed by the application of antiseptic iodine solution. Subcutaneous infiltration anesthesia (4 ml per region of mepivacaine 2% with epinephrine 1 : 100,000) was applied for increased local hemostasis. An incision ~5 cm in length was made through the skin and the muscles and periosteum were elevated to expose the bone. Two pairs of implants (10 × 4 mm, L × Ø) from each of the experimental groups were placed in each tibia according to the manufacturer's instructions under copious sterile saline irrigation and with a torque of about 45 Ncm (last drill 3.5 mm in Ø). The implants were placed with an alternating fashion in terms of medio-distal positioning regarding the group, but with the first group chosen at random. Surgeries were staged between left and right tibia to provide healing times of 2 and 4 weeks, i.e. 12 implants per group per observation time. The first implant was inserted ca. 2 cm below the femoral-tibial-patellar joint line at the central medial-lateral portion of the proximal tibiae. The following implants were placed in a distal direction with inter-implant distances of 1 cm along the central region of the bone until the tibia patellar joint (Fig. 1). Implants were furnished with cover-screws and then soft tissues were sutured in layers with Vicryl 4-0 (Ethicon/Johnson & Johnson®, Miami, FL, USA) and with 4-0 Mononylon Ethicon® (Virbac do Brasil Indústria e Comércio Ltda.) for primary intention healing.

Fig. 1. Two pairs of implants (10 × 4 mm, L × Ø) from each of the experimental groups were placed in each tibia, with an alternating fashion in terms of medio-distal positioning regarding the group, but with the first group chosen at random. Implants were placed with an inter-implant distance of 1 cm. Only one implant per group and observation time was processed for histological and histomorphometrical analysis, i.e. 24 implants are reported herein.

Postoperative pain control was achieved with a single dose of 1 ml pt. 5 kg i.m. Ketoprofen (Ketoprofeno 1% – Laboratórios Vencofarma do Brasil Ltda., Londrina, PR, Brazil). Post-operative infection control was achieved with local application of Rifampin spray (SEM S.A. São Bernardo do Campo, SP, Brazil) and systemic antibiotics (Pentabiotic, 30/0 00 UI/kg i.m.; Fort Dodge Saúde Animal Ltda.) for 7 days. The animals were euthanized under sedation with an anesthesia overdose, and the upper third of the tibias was retrieved. The overlying soft tissues were removed and the specimens were fixed in 10% buffered formalin solution. One implant per group and observation period was processed for biomechanical analysis (results will be reported elsewhere), while the other implant was processed histologically as follows.

Histotechnical procedures and histomorphometry

The specimens (six per group and observation period) were processed for non-decalcified histologic and histomorphometric evaluation. Briefly, bone blocks containing a single implant were produced, rinsed in running water for 24 h, and gradually dehydrated in a series of ascending concentrations of alcohol from 70% to 100% ethanol and embedded in methylmethacrylate. Two to three central longitudinal sections from each implant (~60 µm thick), in a direction perpendicular to the diaphysis, were cut with a free standing sectioning machine (Meprotech, Medeja, Assendelft, Netherlands) and then polished to a final thickness of 24 µm on average with a desktop grinding-polishing unit (Exakt; Exakt Apparatebau GmbH, Norderstedt, Germany). Finally, the sections were stained alternately with toluidine blue or van Gieson's picchio fuchsin. The histological and histomorphometrical evaluation was performed with an incandescent light microscope

(Nikon Corporation Instruments Company®, Kawasaki, Kanagawa, Japan; Leica DM2500M) connected to a computer with dedicated software (Imaging Software NIS-Elements, Basic Research, Nikon®).

For the analysis, the two technically best sections from each specimen were used. In order to exclude any influence on the evaluated parameters from differences in implant site preparation depth and/or implant insertion level, only a central aspect of the implant with the surrounding tissues within the lumen, i.e. excluding the portion of the implant within the cortex, was used in the histomorphometrical evaluation. First, a rectangular area of interest (AOI), 6 mm long and extending 0.5 mm on each side of the pitch of the 4th implant thread was superimposed over the implant image parallel to its long axis and just below the cortical bone (Fig. 2). The AOI included a net of eight vertical (i.e. parallel to the implant axis) and 26 horizontal (i.e. perpendicular to

the implant axis) equidistant lines. Mineralized BIC was evaluated by counting the number of horizontal lines "hitting" the implant where bone was in direct contact with it, expressed as a percentage of the total number of horizontal line-implant intersections. Bone density was evaluated by counting the number of horizontal-vertical line intersections (points) over mineralized bone, expressed as a percentage of the total number of points within the AOI, but excluding the points over the implant.

Statistics

The average of the values from the two sections was used to represent each implant. Data were expressed as mean ± SD and median for each group and observation time. Differences were tested for significance with the Wilcoxon signed-rank test. To evaluate the possible impact of the variation in the shape of the tibia (triangular or oval), depending on the medial-distal position of the implant site, on BIC and BD, simple logistic regression analysis was performed. Possible differences between the groups, regarding the distribution of implants placed sites where the tibia had a triangular and oval shape, were evaluated with a chi-square test. Duplicate measurements made on the sections from eight implants, with a 1-week interval, were used to test for reproducibility also using the Wilcoxon signed-rank test.

Sample size was based on information from previous studies using the same model. A *post-hoc* analysis was done based on the results observed herein to define the minimum difference in BIC between groups that would have been possible to observe with a

Fig. 2. A rectangular area of interest (AOI; in yellow), 6 mm long and extending 0.5 mm from the pitch of the fourth implant thread was superimposed over the image; the rectangle was placed parallel to the long axis of the implant and just below the cortical bone [a]. The AOI included a net of eight vertical and 26 horizontal equidistant lines [b]. Bone-to-implant contact was evaluated by counting the number of horizontal lines "hitting" the implant where bone was in direct contact with it. Bone density was evaluated by counting the number of horizontal-vertical line intersections (points) over mineralized bone [a: overview at ×20 and b: high magnification at ×200].

power of 80% and an alpha error of 0.05%. The level of significance was set to $P < 0.05$, and the IBM SPSS Statistics software was used (IBM Company Inc., Chicago, IL, USA).

Results

All surgical procedures and the subsequent healing period progressed uneventfully. In general, new bone formation along, and in contact with, the implant surface could be observed irrespective of the experimental group and observation period, however, with some variation among implants. Some variation was observed regarding the depth of implant site preparation and implant insertion level, as well as in regard with the shape of the tibia (triangular or oval) at the site of implantation (Fig. 3). Occasionally, the drill had apparently progressed during preparation into or beyond the cortex of the posterior aspect of the tibia, and sometimes the implant was placed deeper (i.e., the implant shoulder was lower than the surface of the tibia). In such instances, increased amounts of bone formation were observed in the posterior aspects of the tibia both at the outer cortex and inside the lumen (i.e., at the apical aspect of the implant). In a few instances, extended new bone formation was observed in the outer aspect of the cortex, apparently because of the elevation of the periosteum during surgery (Fig. 3). At the 2-week observation period, slender woven bone trabeculae had formed in contact with a portion of the implant surface and in continuation with the inner cortex of the tibia (Fig. 4a and b). After 4 weeks of healing, a large portion of the implant surface was in contact with bone and trabecular bone filled a large portion of the lumen itself (Fig. 4c and d). Most of the bone was woven but small quantities of lamellar bone, mainly in close proximity to the cortex could also be observed. In cases where – due to the anatomy of the tibia at the respective site – a large part of the implant was in close proximity to the inner aspect of the cortex, reduced bone formation was observed at 2 weeks. After 4 weeks, however, the extent of bone formation appeared similar to that in the rest of the specimens.

The results of the histomorphometric evaluation are presented in Table 1 and Fig. 5a and b. Similar amounts of BIC and BD were observed in both groups [SAE-HD vs. SAE] at each observation time (Table 1), and there were no statistically significant differences regarding BIC and BD between the groups at

Fig. 3. Representative microhistographs from all implants at 2- and 4 weeks. Please note the variation in the shape of the tibia (oval vs. triangle), depending on the medial-distal position of the implant site. Van Gieson's picro-haematoxylin and toluidine blue stain.

Fig. 4. Representative microhistographs [overview at $\times 20$ and high magnification at $\times 200$] of Hydrophilic SAE-HD [a & c] and etched SAE implants [b & d] at 2 weeks (upper row) and 4 weeks (lower row). No remarkable differences can be observed between groups and between observation periods regarding mineralized bone-to-implant contact and bone density. Most of the bone was woven but small quantities of lamellar bone could also be observed, mainly at the 4-week observation time (Van Gieson stain).

Table 1. Mean $\pm$ SD, median and 95% confidence interval of mineralized bone-to-implant contact (in %) and bone density for SAE-HD and SAE implants at 2- and 4-weeks post-operatively. Differences were tested for statistical significance with the Wilcoxon test

Time <i>in vivo</i>	SAE-HD				SAE				P value
	Mean $\pm$ SD	Median	95% lower	95% upper	Mean $\pm$ SD	Median	95% lower	95% upper	
BIC (%)									
2 weeks	19.57 $\pm$ 13.57	16.63	5.32	33.81	20.33 $\pm$ 7.99	19.05	11.94	28.71	0.75
4 weeks	42.80 $\pm$ 14.48	40.95	27.62	58.31	40.25 $\pm$ 9.45	36.48	30.33	50.17	0.65
BD (%)									
2 weeks	24.85 $\pm$ 16.31	21.75	7.73	41.97	25.66 $\pm$ 8.588	24.65	16.65	34.67	0.34
4 weeks	44.13 $\pm$ 6.461	42.40	37.35	50.91	40.13 $\pm$ 6.461	40.38	35.38	44.87	0.25

Fig. 5. Dot plots of the data on mineralized bone-to-implant contact (a) and bone density (b) at both observation periods ($P > 0.05$). Horizontal lines indicate the mean value and vertical lines indicate the standard deviation.

any observation period (Fig. 5a and b). Regarding the possible impact of the shape of the tibia at the implant site on the results, the simple logistic regression analysis indicated that implants inserted in sites where the tibia has a triangular shape showed significantly higher BIC values ($P = 0.02$), but lower BD values ($P = 0.01$), comparing to implants inserted in sites where the tibia has an oval shape (Fig. S1). However, no significant difference was observed regarding the distribution of implants placed in triangular and oval sites between the SAE-HD and SAE groups neither at the 2-week or the 4-week observation time ($P = 0.88$ and $P = 1.00$ respectively) (Table 2). Based on the observed results in the SAE group, only a rather large difference in BIC (37%) between the two groups would have been possible to disclose with enough power (80%) and the current sample size.

Furthermore, reproducibility analysis showed no significant differences between the two sets of measurements (Table S1).

Discussion

The results of this study have shown that implants with a sandblasted and dual SAE, specifically treated to increase its hydrophilicity (SAE-HD), achieved similar amounts of BIC and peri-implant BD as only sandblasted and dual acid-etched implants (SAE), during early stages of healing.

These findings are in contrast with the results of most of the previously published preclinical *in vivo* studies evaluating hydrophilic implant surfaces (Buser et al. 2004; Bornstein et al. 2008; Rossi et al. 2014; Mainetti et al. 2015) and humans (Bosshardt et al. 2011; Lang et al. 2011). In those studies, implants with chemically modified surfaces with increased hydrophilicity – but different macro- and microstructure surface than the implants used herein – showed improved performance over relevant control implants. For example, in an experiment conducted by Buser et al. (2004), where hydrophilic moderately rough implants

(SLActive®, Straumann Institute, Basel, Switzerland) were inserted in the maxilla of miniature pigs and compared with similar SLA implants – but without the specific surface treatment to increase hydrophilicity – higher amounts of BIC were observed at the hydrophilic implants after 2, 4, and 8 weeks of healing. Similarly, in another study in dogs, SLActive implants showed greater amount of newly formed bone in contact with the implant in comparison with the conventional SLA after 2 weeks of healing; however, after 8 weeks of healing similar amounts of BIC were observed at both types of implants (Bornstein et al. 2008).

As mentioned earlier, hydrophilic surface modification has been shown to achieve rapid coverage of the implant surface with blood, enhance clot formation, adhesion, and differentiation of osteogenic cells (Buser et al. 2004; Masaki et al. 2005; Zhao et al. 2005; Schwarz et al. 2007; Mamalis & Silvestros 2011), upregulate expression of various growth factors, e.g. VEGF-A, EGF (An et al. 2010; Raines et al. 2010), and modulate pro-inflammatory cytokine genes (Hamlet et al. 2012). In a recent study in rabbits, a new commercially available dental implant (Acqua®, Neodent, Curitiba, Brazil) manufactured by an almost identical technique as the SAE-HD herein was shown to possess a significantly higher hydrophilicity comparing to a commercially available implant with surface technology identical with the SAE implant (Neoporus®, Neodent, Curitiba, Brazil) [contact angle $>90^\circ$ vs. $<5^\circ$, respectively] (Sartoretto et al. 2015). Furthermore, “hydrophilic” implants placed in the tibia of the rabbits achieved 1.5 times more BIC compared with the “hydrophobic” ones after 28 days of healing (Sartoretto et al. 2015). Comparison of BIC values among studies evaluating different hydrophilic technologies should of course be made with care, unless the studies include direct evaluation between specific surface technologies; surface chemistry at implants can indeed be easily changed by altering surface roughness (Wennerberg & Albrektsson 2009). Also, dif-

Table 2. Distribution of implants placed in sites where the tibia had a triangular or oval shape in the SAE-HD and SAE groups, at 2- and 4 weeks (evaluated with Chi-square test)

Group	SAE-HD	SAE	P
2 weeks			
Oval (n)	3	2	0.88
Triangular (n)	3	4	
4 weeks			
Oval (n)	2	2	1.00
Triangular (n)	4	4	

ferences in BIC values among studies may relate to differences in the experimental model used, regarding – among other factors – the species, BD in the site of implantation, healing time, and method for histomorphometric estimation. In perspective, lack of significant differences between the SAE-HD and SAE implants in the present study seems unlikely to be due to lack of significant hydrophilicity of this specific manufacturing technology, although this possibility cannot exclusively be rejected.

In a recent systematic review of human histological studies on molecular aspects of early stages of osseointegration (Shanbhag et al. 2015) it has been confirmed that chemical modifications of moderately rough implant surfaces resulting in increased hydrophilicity, indeed seem to enhance molecular processes related to osseointegration during the early stages of wound healing in the clinic. For example, specifically designed titanium devices with SLActive surface, implanted in the retromolar area in humans, achieved significantly higher amounts of BIC after 2- and 4 weeks comparing to devices with SLA surface; after 42 days, however, these differences were no longer evident (Lang et al. 2011). Thus, a possible explanation for the lack of significant differences between the two groups herein could be the fact that the effect of hydrophilicity was unfolded during the very early periods of wound healing, i.e. before the first evaluation time-point of 2 weeks in the present study.

Indeed, as mentioned above, studies providing histological evidence have reported that the advantage of hydrophilic implants was possible to observe only during the early stages of osseointegration, but “disappeared” at later stages, with control implants achieving similar amounts of BIC (Bornstein et al. 2008; Lang et al. 2011). In fact, studies using the same animal model (i.e., tibia of dogs), did not see any differences in terms of osseointegration of titanium implants with different surface technologies after healing times of 2- and 4-weeks (Marin et al. 2008; Bonfante et al. 2011). In the present study, implants from the two groups, were placed randomly in terms of medial-distal position, to avoid any bias from possible differences in the anatomy of the tibia; the tibia of the dog, in a cross-section, has a more triangular shape at its distal aspect close to the epiphysis, while it is more oval more medially toward the diaphysis. The sub-analysis performed in the present study, indeed showed larger amounts of BIC at implants placed toward the distal (triangular) aspect of the tibia comparing to those placed toward the diaphysis (oval). Nevertheless, no differences were observed regarding the medio-distal distribution of the implants in the two groups, and thus lack of differences in terms of BIC and/or BD between groups cannot be attributed to uneven distribution of the medio-distal positioning of implants in the two groups. In context, the tibia of the dog is a rather infrequent testing site; the majority of studies

evaluating implant integration using the dog as the model animal, involves the mandible (Stadlinger et al. 2012). The tibia, however, due to the presence of the large lumen, offers better discriminating capacities than the rich in trabecular bone mandible, for evaluating the potential of surfaces to enhance osseointegration significantly. In this context, based on the observed results in the SAE group, only a rather large difference in BIC (37%) between the two groups would have been possible to disclose with enough power (80%) and the current sample size.

In conclusion, BIC and BD increased with time in both SAE-HD and SAE implants. No significant differences were observed between the two different implant surfaces for any of the evaluated parameters and at any observation time-point.

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References

- Albrektsson, T. & Wennerberg, A. (2004) Oral implant surfaces: Part 1 – review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. *International Journal of Prosthodontics* 17: 536–543.
- Al-Nawas, B., Hangen, U., Duschner, H., Krummner, F. & Wagner, W. (2007) Turned, machined versus double-etched dental implants in vivo. *Clinical Implants Dentistry and Related Research* 9: 71–78.
- An, N., Schedle, A., Wieland, M., Andrukhov, O., Matejka, M. & Rausch-Fan, X. (2010) Proliferation, behavior, and cytokine gene expression of human umbilical vascular endothelial cells in response to different titanium surfaces. *Journal of Biomedical Materials Research Part A* 93: 364–372.
- Balshi, S.F., Wolfinger, G.J. & Balshi, T.J. (2005) A prospective study of immediate functional loading, following the Teeth in a Day protocol: a case series of 55 consecutive edentulous maxillas. *Clinical Implant Dentistry Related Research* 7: 24–31.
- Barewal, R.M., Stanford, C. & Weesner, T.C. (2012) A randomized controlled clinical trial comparing the effects of three loading protocols on dental implant stability. *The International Journal of Oral & Maxillofacial Implants* 27: 945–956.
- Berglundh, T., Stavropoulos, A. & Working Group 1 of the VIII European Workshop on Periodontology (2012) Preclinical in vivo research in implant dentistry. Consensus of the eighth European workshop on periodontology. *Journal of Clinical Periodontology* 12: 1–5.
- Bonfante, E.A., Granato, R., Marin, C., Suzuki, M., Oliveira, S.R., Giro, G. & Coelho, P.G. (2011) Early bone healing and biomechanical fixation of dual acid-etched and as-machined implant with healing chambers: an experimental study in dogs. *The International Journal of Oral and Maxillofacial Implants* 26: 75–82.
- Bornstein, M.M., Valderrama, P., Jones, A.A., Wilson, T.G., Seibl, R. & Cochran, D.L. (2008) Bone apposition around two different sandblasted and acid-etched titanium implant surfaces: a histomorphometric study in canine mandibles. *Clinical Oral Implants Research* 19: 233–241.
- Bosshardt, D.D., Salvi, G.E., Huynh-Ha, G., Ivanovski, S., Donos, N. & Lang, N.P. (2011) The hole of bone debris in early healing adjacent to hydrophilic and hydrophobic implant surfaces in man. *Clinical Oral Implants Research* 22: 357–364.
- Buser, D., Broggini, N. & Wieland, M. (2004) Enhanced bone apposition to a chemically modified SLA titanium surface. *Journal of Dental Research* 83: 529–533.
- Buser, D., Nydegger, T., Oxland, T., Cochran, D.L., Schenk, R.K., Hirt, H.P., Snétivy, D. & Nolte, L.P. (1999) Interface shear strength of titanium implants with a sandblasted and acid-etched surface: a biomechanical study in the maxilla of miniature pigs. *Journal of Biomedical Materials Research* 45: 75–83.
- Chambrone, L., Shibli, J.A., Mercúrio, C.E., Cardoso, B. & Preshaw, P.M. (2015) Efficacy of standard (SLA) and modified sandblasted and acid-etched (SLActive) dental implants in promoting immediate and/or early occlusal loading protocols: a systematic review of prospective studies. *Clinical Oral Implants Research* 26: 359–370.
- Cochran, D.L., Schenk, R.K., Lussi, A., Higginbottom, F.L. & Buser, D. (1998) Bone response to unloaded and loaded titanium implants with a sandblasted and acid-etched surface: a histometric

- study in the canine mandible. *Journal of Biomedical Materials Research* 40: 1–11.
- Derks, J., Häkansson, J., Wennström, J.L., Tomasi, C., Larsson, M. & Berglundh, T. (2015) Effectiveness of implant therapy analysed in a Swedish population: early and late implant loss. *Journal of Dental Research* 94: 545–515.
- Dohan Ehrenfest, D.M., Coelho, P.G., Kang, B.S., Sul, Y.T. & Albrektsson, T. (2010) Classification of osseointegrated implant surfaces: materials, chemistry and topography. *Trends in Biotechnology* 28: 198–206.
- Elias, C.N. & Meirelles, L. (2010) Improving osseointegration of dental implants. *Expert Review of Medical Devices* 7: 241–256.
- Ferrigno, N., Laureti, M., Fanali, S. & Grippaudo, G. (2002) A long-term follow-up study of non-submerged ITI implants in the treatment of totally edentulous jaws. Part I: Ten-year life table analysis of a prospective multicenter study with 1286 implants. *Clinical Oral Implants Research* 13: 260–273.
- Gomes, J.B., Campos, F.E., Marin, C., Teixeira, H.S., Bonfante, E.A., Suzuki, M., Wittek, L., Zanetta-Barbosa, D. & Coelho, P.G. (2013) Implant biomechanical stability variation at early implantation times in vivo: an experimental study in dogs. *The International Journal of Oral and Maxillofacial Implants* 28: 128–134.
- Hamlet, S., Alfarsi, M., George, R. & Ivanovski, S. (2012) The effect of hydrophilic titanium surface modification on macrophage inflammatory cytokine gene expression. *Clinical Oral Implants Research* 23: 584–590.
- Jimbo, R. & Albrektsson, T. (2015) Long-term clinical success of minimally and moderately rough oral implants: a review of 71 studies with 5 years or more of follow-up. *Implant Dentistry* 24: 62–69.
- Khang, W., Feldman, S., Hawley, C.E. & Gunsolley, J. (2001) A multi-center study comparing dual acid-etched and machined-surfaced implants in various bone qualities. *Journal of Periodontology* 72: 1384–1390.
- Kim, Y.K., Kim, J.H., Yi, Y.J., Kwon, M.J. & Yun, P.Y. (2015) Prospective comparative study of tapered implants with SLA surfaces in the maxillary posterior area according to 3- and 6-month loading time. *International Journal of Periodontics Restorative Dentistry* 35: 271–276.
- Lang, N.P., Salvi, G.E., Huynh-Ba, G., Ivanovski, S., Donos, N. & Bosshardt, D.D. (2011) Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. *Clinical Oral Implants Research* 22: 349–356.
- Li, D., Ferguson, S.J., Beutler, T., Cochran, D.L., Sittig, C., Hirt, H.P. & Buser, D. (2002) Biomechanical comparison of the sandblasted and acid-etched and the machined and acid-etched titanium surface for dental implants. *Journal of Biomedical Materials Research* 60: 325–332.
- Mainetti, T., Lang, N.P., Bengazi, F., Sbriccoli, L., Soto Cantero, L. & Botticelli, D. (2015) Immediate loading of implants installed in a healed alveolar bony ridge or immediately after tooth extraction: an experimental study in dogs. *Clinical Oral Implants Research* 26: 435–241.
- Mamalis, A.A. & Silvestros, S.S. (2011) Analysis of osteoblastic gene expression in the early human mesenchymal cell response to a chemically modified implant surface: an in vitro study. *Clinical Oral Implants Research* 22: 530–537.
- Marin, C., Granato, R., Suzuki, M., Gil, J.N., Piatelli, A. & Coelho, P.G. (2008) Removal torque and histomorphometric evaluation of bioceramic grit-blasted/acid-etched and dual acid-etched implant surfaces: an experimental study in dogs. *Journal of Periodontology* 79: 1942–1949.
- Masaki, C., Schneider, G.B., Zaharias, R., Seabold, D. & Stanford, C. (2005) Effects of implant surface microtopography on osteoblast gene expression. *Clinical Oral Implants Research* 16: 650–656.
- Meijer, H.J., Raghoobar, G.M., Batenburg, R.H. & Vissink, A. (2009) Mandibular overdentures supported by two Brånemark, IMZ or ITI implants: a ten year prospective randomized study. *Journal of Clinical Periodontology* 36: 799–806.
- Quinlan, P., Nummikoski, P., Schenk, R., Cagna, D., Mellonig, J., Higginbottom, F., Lang, K., Buser, D. & Cochran, D. (2005) Immediate and early loading of SLA ITI single-tooth implants: an in vivo study. *The International Journal of Oral & Maxillofacial Implants* 20: 360–370.
- Raines, A.L., Olivares-Navarette, R., Wieland, M., Cochran, D.L., Schwartz, Z. & Boyan, B.D. (2010) Regulation of angiogenesis during osseointegration by titanium surface microstructure and energy. *Biomaterials* 31: 4909–4917.
- Rossi, L., Lang, N.P., De Santis, E., Morelli, F., Favero, G. & Botticelli, D. (2014) Bone-healing pattern at the surface of titanium implants: an experimental study in the dog. *Clinical Oral Implants Research* 25: 124–131.
- Rupp, F., Scheideler, L., Olshanska, N., de Wild, M., Wieland, M. & Geis-Gerstorf, J. (2006) Enhancing surface free energy and hydrophilicity through chemical modification of microstructured titanium implant surfaces. *Journal of Biomedical Materials Research Part A* 76: 323–334.
- Sartoretto, S.C., Alves, A.T., Resende, R.F., Calasans-Maia, J., Granjeiro, J.M. & Calasans-Maia, M.D. (2015) Early osseointegration driven by the surface chemistry and wettability of dental implants. *Journal of Applied Oral Science* 23: 279–287.
- Schwarz, F., Ferrari, D., Herten, M., Mihatovic, I., Wieland, M., Sager, M. & Becker, J. (2007) Effects of surface hydrophilicity and microtopography on early stages of soft and hard tissue integration at non-submerged titanium implants: an immunohistochemical study in dogs. *Journal of Periodontology* 78: 2171–2184.
- Schwarz, F., Sager, M., Ferrari, D., Herten, M., Wieland, M. & Becker, J. (2008) Bone regeneration in dehiscence-type defects at non-submerged and submerged chemically modified (SLActive) and conventional SLA titanium implants: an immunohistochemical study in dogs. *Journal of Clinical Periodontology* 35: 64–75.
- Schwarz, F., Wieland, M., Schwartz, Z., Zhao, G., Rupp, F., Geis-Gerstorf, J., Schedle, A., Brogini, N., Bornstein, M.M., Buser, D., Ferguson, S.J., Becker, J., Boyan, B.D. & Cochran, D.L. (2009) Potential of chemically modified hydrophilic surface characteristics to support tissue integration of titanium dental implants. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 88: 544–557.
- Shanbhag, S., Shanbhag, V. & Stavropoulos, A. (2015) Genomic analyses of early peri-implant bone healing in humans: a systematic review. *International Journal of Implant Dentistry* 1: 1–12.
- Stadlinger, B., Pourmand, P., Locher, M.C. & Schulz, M.C. (2012) Systematic review of animal models for the study of implant integration, assessing the influence of material, surface and design. *Journal of Clinical Periodontology* 39(Suppl. 12): 28–36.
- Vasak, C., Busenlechner, D., Schwarze, U.Y., Leitner, H.F., Munoz Guzon, F., Hefti, T., Schlottig, F. & Gruber, R. (2013) Early bone apposition to hydrophilic and hydrophobic titanium implant surfaces: a histologic and histomorphometric study in minipigs. *Clinical Oral Implants Research* 10: 1378–1385.
- Wennerberg, A. & Albrektsson, T. (2009) Effects of titanium surface topography on bone integration: a systematic review. *Clinical Oral Implants Research* 20: 172–184.
- Wennerberg, A. & Albrektsson, T. (2010) On implant surfaces: a review of current knowledge and opinions. *The International Journal of Oral & Maxillofacial Implants* 25: 63–74.
- Wennerberg, A., Galli, S. & Albrektsson, T. (2011) Current knowledge about the hydrophilic and nanostructured SLActive surface. *Clinical, Cosmetic and Investigational Dentistry* 3: 59–67.
- Zhao, G., Schwarz, Z., Wieland, M., Rupp, F., Geis-Gerstorf, J., Cochran, D.L. & Boyan, B.D. (2005) High surface energy enhances cell response to titanium substrate microstructure. *Journal of Biomedical Materials Research Part A* 74: 49–58.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Simple logistic regression analysis indicated that implants inserted in sites where the tibia has a triangular shape showed significantly higher bone-to-implant contact values ($P = 0.02$), but lower bone density values ($P = 0.01$), comparing to implants inserted in sites where the tibia has an oval shape.

Table S1. Results of the reproducibility evaluation showed no significant differences ($P > 0.05$) between the two sets of measurements regarding all evaluated parameters (evaluated with the Wilcoxon signed-rank test for paired observations).

II

RESEARCH

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Interfacial biomechanical properties of a dual acid-etched versus a chemically modified hydrophilic dual acid-etched implant surface: an experimental study in Beagles

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Abstract

Background: The high survival clinical success rates of osseointegration are requisites for establishing a long-term biomechanical fixation and load-bearing potential of endosseous oral implants. The objective of this preclinical animal study was to evaluate the effect of surface microtopography and chemistry on the early stages of biomechanical rigidity with a sandblasted, dual acid-etched surface, with or without an additional chemical modification (SAE-HD and SAE, respectively), in the tibia of Beagle dogs.

Methods: Two pairs of implants, with the same macrogeometry but different surface technology ((a) dual acid-etched surface treatment with hydrochloric and sulfuric acid followed by microwave treatment and insertion in isotonic saline solution to increase hydrophilicity (SAE-HD) (test, $n = 12$) and (b) dual acid-etched surface (SAE) (control, $n = 12$)), were installed bilaterally in the proximal tibia of six Beagle dogs. In order to determine the effect of surface modification on biomechanical fixation, a test protocol was established to assess the torque and a complete set of intrinsic properties. Maximum removal torque (in N cm) was the primary outcome measure, while connection stiffness (N cm/rad) and removal energy ($\times 10^{-2}$ J) were the secondary outcome measures and were assessed after 2 and 4 weeks in vivo. A general linear statistical model was used and performed for significant differences with the one-way ANOVA followed by Tukey post hoc test ($P < 0.05$).

Results: The removal torque values did not reveal significant statistical differences between SAE-HD and SAE implants at any observation times ($P = 0.06$). Although a slight increase over time could be observed in both test and control groups. SAE-HD showed higher removal energy at 4 weeks ($999.35 \pm 924.94 \times 10^{-2}$ J) compared to that at 2 weeks ($421.94 \pm 450.58 \times 10^{-2}$ J), while SAE displayed lower values at the respective healing periods ($P = 0.16$). Regarding connection stiffness, there were no significant statistical differences neither within the groups nor over time. There was a strong, positive monotonic correlation between removal torque and removal energy ($=0.722$, $n = 19$, $P < 0.001$).

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Conclusions: In this study, no significant differences were observed between the specific hydrophilic (SAE-HD) and hydrophobic (SAE) surfaces evaluated, in terms of biomechanical properties during the early osseointegration period.

Keywords: Biomaterial, Dental implant, Removal torque, Removal energy, Connection stiffness, Implant roughness, Wettability, Dogs, New methodology assessment for implants

Background

The progressive evolution of oral implant surface technology (i.e., micro to nanotopography and chemical composition) [1, 2], implant macrogeometry, surgical procedures [3–5], and loading protocols [6–8] has resulted in high survival and clinical success rates [9]. Accordingly, chemically active micro and nanostructured implant surfaces, presenting moderate surface roughness (R_a/S_a values between 1 and 2 μm), enhance host-to-implant interactions [10–12] and have been shown to shorten the period needed for time-critical functional implant loading (i.e., so-called immediate or early loading) [13–15].

Recent *in vitro* analyses support the concept that hydrophilic surfaces upregulate the expression of angiogenic factors, activate the production of anti-inflammatory factors, and downregulate the expression of pro-inflammatory cytokines by osteoblasts [16] and macrophage-like cells [17, 18], and regulate osteogenic differentiation and maturation of mesenchymal stem cells (MSCs) [10, 19, 20] and human osteoblast-like cells [21–23], increasing osteogenesis and decreasing osteoclastogenesis [11, 23, 24]. Furthermore, higher surface energy and hydrophilicity is demonstrated to induce faster bone-to-implant contact (%BIC) and bone density, both in preclinical *in vivo* experiments [25–27] and in patients [7, 28, 29]. Indeed, higher biomechanical stability as expression of primary and secondary bone anchorage is recorded following hydrophilic implant placement [26, 30].

Particularly, a superhydrophilic moderately rough titanium (Ti) implant surface (contact angle less than 5°) has been suggested to play a critical role during the early healing period [27] and establishment of a successful osseointegration [31] in preclinical *in vivo* studies. The microarchitecture and density of the trabecular bone formed around oral implants, characterized by a high ratio of metabolic activity and remodeling, is one of the main determinants of interfacial shear strength, mechanical resistance, and adaptation to overloading stress [32]. The potential of chemically modified superhydrophilic implant surfaces to enhance osteogenic differentiation and increase early bone apposition onto the implant may shorten the implant stability drop, occurring due to bone remodeling during the first few weeks of implantation [13].

Consequently, such microstructured surface technology may lessen the healing (non-loading) period and allow more readily immediate or early functional implant loading in patients with reduced bone density. Nevertheless, there is lack of knowledge regarding the intrinsic biomechanical aspects of osseointegration related to this specific implant surface technology (i.e., removal torque, removal energy, and connection stiffness) during the initial osseointegration process.

Evaluation of biological effects and biomechanical properties of innovative technologies in the field of implant dentistry in preclinical animal models, prior to translational research, complies with standard regulations [33]. Thus, the aim of this preclinical animal study was to evaluate the effect of surface microtopography and chemistry on the biomechanical properties of implants with a sandblasted, dual acid-etched surface, with or without an additional chemical modification (SAE-HD¹ and SAE² respectively), during the early stages of osseointegration in the tibia of Beagle dogs. Specifically, we hypothesized that a chemically active implant surface presenting superhydrophilicity generates higher interfacial shear strength in comparison to a hydrophobic surface, expressed as higher maximum removal torque, removal energy, and connection stiffness.

Methods

The present preclinical *in vivo* study is reported according to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines, in regard with relevant items [33]. The animal experimental protocol was approved by the Bioethics Committee for Animal Experimentation (CEUA, protocol no. 098/10) at the Federal University of Uberlândia and followed the normative guidelines of the National Council for Animal Control and Experimentation (CONCEA), constituent of the Ministry of Science, Technology and Innovation (MCTI), Law no. 11.794, 08/19/2008, Brazil. The *in vivo* part of the study was conducted between November 2012 and January 2013.

Experimental units

Twenty-four commercially pure Ti implants (10 mm $\times$ 4 mm, $L \times \varnothing$) with a moderately rough surface, produced by means of sandblasting and dual acid-etching with

hydrochloric and sulfuric acid were used in the present study. Implants in the control group were only sand-blasted and dual acid-etched (SAE; $n = 12$). In the test group, after sandblasting and dual acid etching, implants received proprietary technology treatment, including microwave treatment and insertion in isotonic saline solution resulting in significantly increased hydrophilicity (SAE-HD; $n = 12$). These specific implant surfaces exhibit the following 3D surface roughness parameters for SAE and SAE-HD, respectively: S_a $1.44 \mu\text{m} \pm 1.15$ and $1.26 \mu\text{m} \pm 0.17$; S_z $14.57 \mu\text{m} \pm 1$ and $16.20 \mu\text{m} \pm 7.8$; S_{dr} 1.51 and 1.21% ; S_{ds} $658.67 \text{ 1/mm}^2 \pm 27.42$ and $643.33 \text{ 1/mm}^2 \pm 37.74$; and S_{sk} -0.51 and -0.43 [27]. The chemical composition of the different surfaces was examined by X-ray photoelectron spectroscopy (XPS). The atomic percentage values of carbon were 49 and 17, and 22 and 59 of oxygen in SAE and SAE-HD groups, respectively. The surface energy and wettability was investigated by means of a static contact angle analysis by means of the sessile drop technique [34] applying simulated body fluid (SBF) solution. SAE-HD disks reveal a superhydrophilic behavior (contact angle $< 5^\circ$), whereas SAE surfaces display superhydrophobic properties (contact angle $> 90^\circ$).

The implants present similar macrodesign with an external hexagon connection system and cylindrical body containing double triangular threads with high potential for bone compression recommended for type III and IV bone, commercially available as Titamax Ti Ex[®]. Neodent[®] supplied all implants.

Animal model

Six male Beagle dogs (~ 1.5 years old), weighting between 13 and 15 kg, were used in the present study. All animals were acclimatized in the experimental animal care facility of Federal University of Uberlândia for 2 weeks previously to the experimental procedures and randomly pair-housed in standard shelters (1×1.5 m kennel) to allow environmental enrichment (i.e., variety of toys, daily group play sessions, resistance running, and training) at the ambient temperature of 22°C , under controlled humidity and 12-h circadian rhythm. The diet consisted of hard pellet and water ad libitum. The animal caretakers were blind to the experimental groups. The level of pain, distress, or suffering was daily assessed during the observation period to ensure the welfare of the animals. Aiming to guarantee selection blindness during the experimental allocation, the groups were systematically coded by a third person and the first implant to be placed in each tibia was randomly assigned. Sample size was calculated based on information in previous studies using the dog tibia model to allow evaluation of possible differences in bone-to-implant contact between groups. The surgical procedures and

the histological/histomorphometrical outcomes of the study have been reported elsewhere [31].

Briefly, two pairs of implants ($10 \text{ mm} \times 4 \text{ mm}$, $L \times \varnothing$) from each of the experimental groups were placed under copious sterile saline irrigation and with a torque of about 45 N cm (last drill $3.5 \text{ mm } \varnothing$) in each proximal tibia of the animals (total no. of implants 48). Implants were placed with an alternating fashion in terms of medio-distal positioning with the first group chosen at random and surgeries were staged between left and right tibia to provide 2 and 4 weeks of healing times, i.e., 12 implants per group and per observation time. The first implant was inserted ca. 2 cm below the femoral-tibial-patellar joint line at the central medial-lateral portion of the proximal tibiae (Fig. 1). The following implants were placed in a distal direction with inter-implant distances of 1 cm along the central region of the bone until the tibia patellar joint. Implants were furnished with cover screws and then soft tissues were sutured in layers for primary intention healing. Postoperative pain and infection control was provided for 7 days. The animals were euthanized under sedation with an anesthesia overdose, and the upper third of the tibia was retrieved. The specimens were fixed in 10% buffered formalin solution and half of them were allocated to histomorphometric analysis and the other half to biomechanical analysis, reported herein.

Assessment of interfacial biomechanical properties

To assess the biomechanical strength of the bone-implant interface, the following parameters were assessed: (a) maximum removal torque (N cm) (primary outcome measure), obtained during the unscrewing process (primary outcome measure); (b) connection stiffness (N cm/rad), corresponding to the ratio between

Fig. 1 Two pairs of implants ($10 \text{ mm} \times 4 \text{ mm}$, $L \times \varnothing$) from each of the experimental groups were placed in each tibia with an alternating fashion in terms of medio-distal positioning regarding the group, but with the first group chosen at random. Implants were placed with an inter-implant distance of 1 cm

removal torque and angular displacement (secondary outcome measure); and (c) removal energy ($\times 10^{-2}$ J), corresponding to the energy (workload) necessary to completely unscrew the implant (secondary outcome measure).

The removal torque test was conducted on a Shimadzu universal testing machine.⁴ This equipment was adapted in order to determine the referred properties (Fig. 2a, b). A horizontal shaft, supported by two ball bearings, with Allen keys socket on one end and a rotation sensor on the other end, was connected with a steel string to the mobile span of the Shimadzu universal testing machine, in such a way that the linear motion was converted to a rotational motion. The dog's tibia bone block containing the implant was placed in alignment and inside the Allen keys socket and fixed with an adjustable clamp, in order not to rotate during the test. The upper span speed, at which the string was attached, was adjusted to produce a shaft rotation speed of 0.005 rad/s. During the test, both torque (N cm) and angular displacement (rad) were acquired using a sampling rate of 10 samples/s (Fig. 3a, b). In order to calculate the connection stiffness (N cm/rad), the tangent method was applied to the data after obtaining the linear correlation coefficient (R^2) compared to the secant method, revealing the absence of mathematical discrepancy between the application of both methods (Fig. 4).

Calibration of one blinded examiner (R.N.R.) and repeated measurements for data reproducibility was performed under supervision and prior to performing the removal torque test and respective calculation of connection stiffness.

Statistical analysis

A general linear statistical model with torque, energy, and stiffness as dependent variables and implant surface and time in vivo as independent variables was used at 95% level of significance and performed by one-way ANOVA followed by Tukey's post hoc test. The Spearman rank

correlation test was taken in order to test the association concerning the investigated dependent variables. Since sample size calculation, as already mentioned, was made to allow evaluation of possible differences in bone-to-implant contact between groups, a post hoc analysis was performed to define the minimum detectable difference between groups regarding the parameters assessed herein, with a power of 80% and an alpha error of 0.05%. The IBM® SPSS® Statistics software⁵ was used.

Results

No remarkable events were observed during the surgical procedures and the subsequent healing period. The relative biomechanical performance of both experimental implant surfaces is illustrated in a representative graph of removal torque versus angular displacement. The removal torque, removal energy, and connection stiffness values are described in terms of mean, standard deviation, and 95% confidence interval for mean in Fig. 5. The results of one-way ANOVA variance and Tukey's post hoc test values of the variables for SAE-HD and SAE implant at 2 and 4 weeks postoperatively ($n = 6$) are demonstrated in Table 1.

The removal torque values (N cm) did not reveal significant statistical differences between SAE-HD and SAE implants at any observation period ($P = 0.06$), but a slight increase over time could be observed in both test and control groups (Fig. 5a). Removal torque at 2 weeks was 60.18 ± 24.12 and 54.00 ± 29.91 N cm, and at 4 weeks was 88.11 ± 14.96 and 79.05 ± 14.59 N cm for SAE-HD and SAE implants, respectively (Fig. 5b). SAE-HD showed higher removal energy [J] at 4 weeks (9.99 ± 9.25) compared to 2 weeks (4.22 ± 4.50), while SAE displayed lower values at corresponding healing times (5.21 ± 1.49 vs. 2.83 ± 2.49 , respectively) ($P = 0.16$). Regarding connection stiffness (N cm/rad), SAE showed lower values at 2 weeks (462.57 ± 507.75) compared with 4 weeks of healing (689.01 ± 496.28), however not

Fig. 2 Adaptation of Shimadzu universal testing machine for performing removal torque test of dental implants. **a** General view. **b** Assembly detail of connection between Allen keys socket and the implant placed in the tibia

statistically significant (Fig. 5c). SAE-HD disclosed similar results at 2 and 4 weeks (652.98 ± 111.26 vs. 661.56 ± 369.28 , respectively) without statistically significant differences neither between the groups, nor over time ($P = 0.76$).

A Spearman's correlation test was run to determine the connection between maximum removal torque, removal energy, and connection stiffness values (Table 2) during the interfacial strength assessment. There was a strong, positive monotonic correlation between removal torque and removal energy ($=0.722$, $n = 19$, $P < 0.001$). However, there was no evidence of positive correlation between removal torque and connection stiffness ($=0.352$, $n = 24$, $P = 0.092$). Moreover, a negative correlation between removal energy and connection stiffness was observed ($= -0.91$, $n = 19$, $P = 0.710$).

Discussion

Improving surface wettability aims to increase the implant surface area achieving most favorable protein adsorption and cellular adhesion and thereby to positively regulate the biological response at the initial osseointegration process. Thus, the superior potential of superhydrophilic surfaces in enhancing osseointegration at early stages of bone formation may also enhance their load-bearing capacity and biomechanical resistance.

In the present study, both SAE-HD implants and SAE implants showed relatively high amounts of maximum removal torque values at both observation times. In contrast, the SAE-HD implants showed relatively high values in removal energy compared with SAE implants at both 2 and 4 weeks. Specifically, the test group presented consistently higher values (about 100% higher) in

Fig. 5 Mean and standard deviation of the biomechanical data at both observation periods ($P > 0.05$). **a** Removal torque. **b** Removal energy. **c** Connection stiffness

Table 1 One-way ANOVA variance and Tukey's post hoc test values of removal torque (N cm), removal energy [N cm/rad (0.01 J)], and connection stiffness [N cm/rad] for SAE-HD and SAE implants at 2 and 4 weeks postoperatively (n = 6; P < 0.05)

		Sum of squares	df	Mean square	F	Sig. (P value)
Removal torque	Between groups	4572.571	3	1524.190	3.186	0.06
	Within groups	9569.095	21	478.455		
	Total	14,141.666	24			
Removal energy	Between groups	1,245,769.635	3	415,256.545	1.677	0.16
	Within groups	3,715,217.914	16	247,681.194		
	Total	4,960,987.549	19			
Connection stiffness	Between groups	193,886.351	3	64,628.784	0.396	0.76
	Within groups	3,264,332.301	18	163,216.615		
	Total	3,458,218.652	21			

removal energy compared with the control group at both observation times. Further, SAE-HD implants showed high values in connection stiffness already after 2 weeks of healing, while SAE implants required 4 weeks of healing to reach a similar level. Thus, despite the fact that the differences between the two groups were not significant for any of the evaluated parameters or observation times, the results seem to indicate that differences in surface properties between SAE-HD and SAE implants, somehow influenced osseointegration and intrinsic properties of shear strength. Indeed, greater removal torque values and interfacial stiffness for hydrophilic implants (modSLA)¹ between 2 and 4 weeks, in comparison with SLA², have been previously reported [35]. In this study [35], performed in the anterior maxilla of miniature pigs, hydrophilic implants revealed, on average, 8–21 and 9–14% significantly higher removal torque and interfacial stiffness values, respectively, than those of the SLA implants. Due to the remodeling process, the biomechanical parameters decreased with time for both implant surfaces, reflecting the developing biological stability.

It has been previously reported the existence of a correlation between removal torque and %BIC values [26],

although the nature of these parameters differs from one another (three-dimensional versus most often two-dimensional parameter) [36]. Indeed, the lack of differences between the groups herein reflect well the results of the histomorphometric analysis of the other half of implants in the present study, reported elsewhere [31]. In particular, similar amounts of osseointegration in terms of %BIC and bone density were observed in both groups (SAE-HD vs. SAE) at each observation time, and there were no statistically significant differences regarding the respective parameters between the two observations times [31]. In contrast, Sartoretto et al. [27] demonstrated that Acqua[®] implants (Neodent[®]), which present similar technology as the SAE-HD implants, resulted in accelerated osseointegration when placed in tibia of rabbits after 2 weeks of healing, compared with implants with the Neoporos[®] surface, which in turn presents similar technology with the SAE surface. The difference between the study of de Jesus et al. [31] and Sartoretto et al. [27] in terms of the impact of surface technology on histomorphometric osseointegration parameters may be due to anatomical and/or biological differences in the experimental

Table 2 Spearman rank correlation coefficient values between removal torque (N cm), removal energy [N cm/rad (0.01 J)], and connection stiffness [N cm/rad] for SAE-HD and SAE implants at 2 and 4 weeks postoperatively (n = 6; P < 0.01)

			Removal torque	Removal energy	Connection stiffness
Spearman's rho	Removal torque	Coefficient	1.000	0.722	0.352
		Sig. (two-tailed)		0.000	0.092
		N	24	24	24
	Removal energy	Coefficient	0.722	1.000	(-) 0.091
		Sig. (two-tailed)	0.000		0.710
		N	19	19	19
	Connection stiffness	Coefficient	0.352	(-) 0.091	1.000
		Sig. (two-tailed)	0.092	0.710	
		N	21	21	21

models employed. In this context, although there is no scientific support regarding the optimal experimental model to evaluate aspects of osseointegration, the dog is one of the most commonly used animal platforms [36]. The mandible of dogs is the most frequent location; however, a high percentage of studies on implant integration have used extra-oral implant sites, including the tibia [37]. It is suggested that due to its anatomy, with a large lumen and relatively low trabecular density [38], it possesses high discriminating potential regarding the impact of implant surface technologies to enhance osseointegration. Furthermore, the tibia allows placement of a larger number of implants comparing with the mandible, thus allowing the use of fewer animals and/or multiple types of comparisons/tests (e.g., biomechanical and histological evaluation).

On the other hand, lack of significant differences between the two groups in the present experiment could be due to the fact that the effect of hydrophilicity, in terms of accelerating bone healing and osseointegration, was unfolded before the first evaluation time-point of 2 weeks, i.e., during the very early healing period. Consistently, pre-clinical investigations show the potential of chemically modified surfaces to rapidly modulate the host-to-implant response upon prompt adsorption of blood proteins [20, 22]. In a recent study reported by Vasak et al. [39], hydrophilic implants (SLActive^a) strengthened the apposition of newly formed bone at the very early healing period between 5 and 10 days in the intra-oral model of minipigs, even though they did not reveal significant statistical differences in comparison to hydrophobic surfaces (SLA). Clinically, a systematic review of human histological studies on molecular aspects of osseointegration [29] has shown that moderately rough implant surfaces with high hydrophilicity enhance molecular processes related to osseointegration during the early stages of wound healing. Similar clinical studies assessed the degree of new bone-to-implant contact (%NBIC) around SLActive implants in comparison to SLA placed in the mandibular retromolar region in man during the early stages of osseointegration [28, 40]. The authors reported a progressive increase in %NBIC around both implants, whereas chemically active surfaces disclosed higher values at 2 and 4 weeks compared with SLA, no longer observed after 6 weeks of osseointegration.

Studies including biomechanical analysis of osseointegration are usually assessing maximum removal torque. Although this parameter is important, use of a single parameter appears not sufficient for a complete biomechanical assessment of osseointegration. Herein, connection stiffness, reflecting the rigidity (i.e., the stability of the implant under load), and removal energy, reflecting the overall energy (workload) necessary to loosen the

bone-to-implant connection, were additionally assessed. It is suggested that the use of these three parameters in a complementary manner is essential for complete evaluation of the biomechanical properties of implants. Indeed, two different osseointegrated implants can have the same maximum removal torque, but distinct connection stiffness; similarly, they may show the same removal energy but perform in a very distinct fashion and show distinct connection stiffness and maximum removal torque. To the best of the authors' knowledge, this is the first original research reporting removal energy as an intrinsic removal torque property in relation to connection stiffness during biomechanical assessment of hydrophilic implants; however, removal energy has already been used assessing mini-implants with hydrophobic surfaces [41].

In fact, the observed lack of differences between the groups could be attributed to the relatively low number of specimens per group. According to the observed data herein, applying a high power (80%) with the present sample size would have revealed a relatively large difference in removal torque equivalent to 43% between the experimental groups. Regarding removal energy and connection stiffness, this difference would have been equal to 71 and 34%, respectively. In addition, a significant difference may not have been achieved due to the nature of the specimens. Biological material properties vary greatly from organism to organism and even in the same animal. Therefore, in future studies when using the presented methodology, a greater number of specimens must be considered. This will evidence a superior discrimination capacity. Nonetheless, the current methodology is far more reliable than the use of a single parameter methodology.

Conclusions

No significant differences were observed between the specific hydrophilic (SAE-HD) and hydrophobic (SAE) surfaces evaluated in this study, in terms of biomechanical properties during the early osseointegration period.

Endnotes

¹Chemically modified sandblasted, large grit, and acid-etched surfaces

²Sandblasted, large grit, and acid-etched surfaces

³Neodent, Rua Benjamin Lins, 742, Curitiba, PR 80420-100, Brazil

⁴Shimadzu Corporation, Nishinokyo Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan

⁵IBM Corporation v.23, 1 New Orchard Road, Armonk, New York 10, 504-1722, USA

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RNRJ participated in the study concept and design and performed the animal operations, biomechanical test, and data analysis and interpretation. EC, PVA, and AR contributed to define the test protocol and performed the biomechanical test and data analysis. CCGM and DZ participated in the study concept and design and performed the animal operations and data analysis. AS contributed to data analysis, interpretation, and drafting of the manuscript. All authors have contributed to and/or critically revised the manuscript and given approval to the final version of the manuscript.

Ethics approval

The animal experimental protocol was approved by the Bioethics Committee for Animal Experimentation (CEUA, protocol no. 098/10) at the Federal University of Uberlândia.

Competing interests

Rainde Naiara Rezende de Jesus, Eunice Carrilho, Pedro V. Antunes, Amílcar Ramalho, Camilla Christian Gomes Moura, Andreas Stavropoulos, and Darceny Zanetta-Barbosa declare that they have no competing interests.

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References

- Cochran DL, Jackson JM, Jones AA, Jones JD, Kaiser DA, Taylor TD, et al. A 5-year prospective multicenter clinical trial of non-submerged dental implants with a titanium plasma-sprayed surface in 200 patients. *J Periodontol*. 2011; 82(7):990–9. <https://doi.org/10.1902/jop.2011.100464>.
- Wallkamm B, Ciocco M, Ettlin D, Syfrig B, Abbott W, Listrom R, et al. Three-year outcomes of Straumann Bone Level SLActive dental implants in daily dental practice: a prospective non-interventional study. *Quintessence Int*. 2015;46(7):591–602. <https://doi.org/10.3290/j.gia.34076>.
- Baires-Campos FE, Jimbo R, Bonfante EA, Fonseca-Oliveira MT, Moura C, Zanetta-Barbosa D, et al. Drilling dimension effects in early stages of osseointegration and implant stability in a canine model. *Med Oral Patol Oral Cir Bucal*. 2015;20(4):471–9.
- Blanco J, Alvarez E, Munoz F, Linares A, Cantalapiedra A. Influence on early osseointegration of dental implants installed with two different drilling protocols: a histomorphometric study in rabbit. *Clin Oral Implants Res*. 2011; 22(1):92–9. <https://doi.org/10.1111/j.1600-0501.2010.02009>.
- Jimbo R, Tovar N, Anchieta RB, Machado LS, Marin C, Teixeira HS, et al. The combined effects of undersized drilling and implant macrogeometry on bone healing around dental implants: an experimental study. *Int J Oral Maxillofac Surg*. 2014;43(10):1269–75. <https://doi.org/10.1016/j.ijom.2014.03.017>.
- Marković A, Colić S, Šćepanović M, Mišić T, Dinić A, Bhushal DS, A 1-year prospective clinical and radiographic study of early-loaded bone level implants in the posterior maxilla. *Clin Implant Dent Relat Res*. 2015;17(5): 1004–13. <https://doi.org/10.1111/cid.12201>.
- Nicolau P, Korostoff J, Ganeles J, Jackowski J, Krafft T, Neves M, et al. Immediate and early loading of chemically modified implants in posterior jaws: 3-year results from a prospective randomized multicenter study. *Clin Implant Dent Relat Res*. 2013;15(4):600–12. <https://doi.org/10.1111/j.1708-8208.2011.00418>.
- Romanos G, Grizas E, Laukart E, Nentwig GH. Effects of early moderate loading on implant stability: a retrospective investigation of 634 implants with platform switching and Morse-tapered connections. *Clin Implant Dent Relat Res*. 2016;18(2):301–9. <https://doi.org/10.1111/cid.12314>.
- Karabuda ZC, Abdel-Haq J, Arsan V. Stability, marginal bone loss and survival of standard and modified sand-blasted, acid-etched implants in bilateral edentulous spaces: a prospective 15-month evaluation. *Clin Oral Implants Res*. 2011;22(8):840–9. <https://doi.org/10.1111/j.1600-0501.2010.02065>.
- Wall I, Donos N, Carlqvist K, Jones F, Brett P. Modified titanium surfaces promote accelerated osteogenic differentiation of mesenchymal stromal cells in vitro. *Bone*. 2009;45(1):17–26. <https://doi.org/10.1016/j.bone.2009.03.662>.
- Mamalis AA, Markopoulou C, Vrotsos I, Koutsilieris M. Chemical modification of an implant surface increases osteogenesis and simultaneously reduces osteoclastogenesis: an in vitro study. *Clin Oral Implants Res*. 2011;22(6):619–26. <https://doi.org/10.1111/j.1600-0501.2010.02027>.
- Lotz EM, Olivares-Nasarette R, Berner S, Boyan BD, Schwartz Z. Osteogenic response of human MSCs and osteoblasts to hydrophilic and hydrophobic nanostructured titanium implant surfaces. *J Biomed Mater Res A*. 2016; 104(12):3137–48. <https://doi.org/10.1002/jbma.35852>.
- Chambre L, Shibli JA, Mercurio CE, Cardoso B, Preshaw PM. Efficacy of standard (SLA) and modified sandblasted and acid-etched (SLActive) dental implants in promoting immediate and/or early occlusal loading protocols: a systematic review of prospective studies. *Clin Oral Implants Res*. 2015;26(4): 359–70. <https://doi.org/10.1111/clr.12347>.
- Heinemann F, Hasan I, Bourauel C, Biffar R, Mundt T. Bone stability around dental implants: treatment related factors. *Ann Anat*. 2015;199:3–8. <https://doi.org/10.1016/j.aanat.2015.02.004>.
- Hinkle RM, Rimer SR, Morgan MH, Zeman P. Loading of titanium implants with hydrophilic endosteal surface 3 weeks after insertion: clinical and radiological outcome of a 12-month prospective clinical trial. *J Oral Maxillofac Surg*. 2014;72(8):1495–502. <https://doi.org/10.1016/j.joms.2014.04.016>.
- Hyzy SL, Olivares-Navarette R, Hutton DL, Tan C, Boyan BD, Schwartz Z. Microstructured titanium regulates interleukin production by osteoblasts, an effect modulated by exogenous BMP-2. *Acta Biomater*. 2013;9(3):5821–9. <https://doi.org/10.1016/j.actbio.2012.10.030>.
- Hamlet S, Alfarsi M, George R, Ivanovski S. The effect of hydrophilic titanium surface modification on macrophage inflammatory cytokine gene expression. *Clin Oral Implants Res*. 2012;23(5):584–90. <https://doi.org/10.1111/j.1600-0501.2011.02325>.
- Hotchkiss KM, Ayad NB, Hyzy SL, Boyan BD, Olivares-Navarette R. Dental implant surface chemistry and energy alter macrophage activation in vitro. *Clin Oral Implants Res*. 2017;28(4):414–23. <https://doi.org/10.1111/clr.12814>.
- Rosa AL, Kato RB, Castro Raucci LMS, Teixeira LN, de Oliveira FS, Bellesini LS, et al. Nanotopography drives stem cell fate toward osteoblast differentiation through $\alpha 1 \beta 1$ integrin signaling pathway. *J Cell Biochem*. 2014;115(3):540–8. <https://doi.org/10.1002/jcb.24688>.
- Boyan BD, Cheng A, Olivares-Navarette R, Schwartz Z. Implant surface design regulates mesenchymal stem cell differentiation and maturation. *Adv Dent Res*. 2016;28(1):10–7. <https://doi.org/10.1177/0022034515624444>.
- Mamalis AA, Silvestros SS. Analysis of osteoblastic gene expression in the early human mesenchymal cell response to a chemically modified implant surface: an in vitro study. *Clin Oral Implants Res*. 2011;22(5):530–7. <https://doi.org/10.1111/j.1600-0501.2010.02049>.
- Kato RB, Roy B, De Oliveira FS, Ferraz EP, De Oliveira PT, Kemper AG, et al. Nanotopography directs mesenchymal stem cells to osteoblast lineage through regulation of microRNA-SMAD-BMP-2 circuit. *J Cell Physiol*. 2014; 229(11):1690–6. <https://doi.org/10.1002/jcp.24614>.
- Zhao G, Schwartz Z, Wieland M, Rupp F, Geis-Gerstoffer J, Cochran DL, et al. High surface energy enhances cell response to titanium substrate microstructure. *J Biomed Mater Res A*. 2005;74(1):49–58. <https://doi.org/10.1002/jbma.30320>.

24. Bang SM, Moon HJ, Kwon YD, Yoo JY, Pae A, Kwon IK. Osteoblastic and osteoclastic differentiation on SLA and hydrophilic modified SLA titanium surfaces. *Clin Oral Implants Res.* 2014;25(7):831–7. <https://doi.org/10.1111/cir.12146>.
25. Buser D, Broggini N, Wieland M, Schenk RK, Denzer AJ, Cochran DL, et al. Enhanced bone apposition to a chemically modified SLA titanium surface. *J Dent Res.* 2004;83(7):529–33. <https://doi.org/10.1177/154405910408300704>.
26. Gottlow J, Barkamo S, Sennerby L. An experimental comparison of two different clinically used implant designs and surfaces. *Clin Implant Dent Relat Res.* 2012;14(Suppl 1):204–12. <https://doi.org/10.1111/j.1708-8208.2012.00439>.
27. Sartoretto SC, Alves AT, Resende RF, Calasans-Maia J, Granjeiro JM, Calasans-Maia MD. Early osseointegration driven by the surface chemistry and wettability of dental implants. *J Appl Oral Sci.* 2015;23(3):279–87. <https://doi.org/10.1590/1678-775720140483>.
28. Lang NP, Salvi GE, Huynh-Ba G, Ivanovski S, Donos N, Bosshardt DD. Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. *Clin Oral Implants Res.* 2011;22(4):349–56. <https://doi.org/10.1111/j.1600-0501.2011.02172>.
29. Shanhag S, Shanhag V, Stavropoulos A. Genomic analyses of early peri-implant bone healing in humans: a systematic review. *Int J Implant Dent.* 2015;1(1):5. <https://doi.org/10.1186/s40729-015-0006-2>.
30. Guler AU, Sumer M, Duran I, Sandikci EO, Telcioglu NT. Resonance frequency analysis of 208 Straumann dental implants during the healing period. *J Oral Implantol.* 2013;39(2):161–7. <https://doi.org/10.1563/AAID-JOI-D-11-00060>.
31. de Jesus RNR, Stavropoulos A, Oliveira MTF, Soares PBF, Moura CCG, Zanetta-Barbosa D. Histomorphometric evaluation of a dual acid-etched vs. a chemically modified hydrophilic dual acid-etched implant surface. An experimental study in dogs. *Clin Oral Implants Res.* 2017;28(5):551–7. <https://doi.org/10.1111/cir.12833>.
32. Frost HM. Skeletal structural adaptations to mechanical usage (SATMU): 1. Redefining Wolff's law: the bone modeling problem. *Anat Rec.* 1990;226(4):403–13.
33. Berglundh T, Stavropoulos A; Working Group 1 of the VIII European Workshop on Periodontology. Preclinical in vivo research in implant dentistry. Consensus of the eighth European workshop on periodontology. *J Clin Periodontol* 2012; 39 Suppl 12:1–5. doi: <https://doi.org/10.1111/j.1600-051X.2011.01827>.
34. Sawase T, Jimbo R, Baba K, Shibata Y, Ikeda T, Atsuta M. Photo-induced hydrophilicity enhances initial cell behavior and early bone apposition. *Clin Oral Implants Res.* 2008;19(5):491–6. <https://doi.org/10.1111/j.1600-0501.2007.01509>.
35. Ferguson SJ, Broggini N, Wieland M, de Wild M, Rupp F, Geis-Gerstorfer J, et al. Biomechanical evaluation of the interfacial strength of a chemically modified sandblasted and acid-etched titanium surface. *J Biomed Mater Res A.* 2006;78(2):291–7. <https://doi.org/10.1002/jbm.a.30678>.
36. Marin C, Granato R, Suzuki M, Gil JN, Piattelli A, Coelho PG. Removal torque and histomorphometric evaluation of bioceramic grit-blasted/acid-etched and dual acid-etched implant surfaces: an experimental study in dogs. *J Periodontol.* 2008;79(10):1942–9. <https://doi.org/10.1902/jop.2008.080106>.
37. Stadlinger B, Pourmand P, Locher MC, Schulz MC. Systematic review of animal models for the study of implant integration, assessing the influence of material, surface and design. *J Clin Periodontol.* 2012;39(Suppl 12):28–36. <https://doi.org/10.1111/j.1600-051X.2011.01835>.
38. Pearce AJ, Richards RG, Milz S, Schneider E, Pearce SG. Animal models for implant biomaterial research in bone: a review. *Eur Cell Mater.* 2007;13(1):1–10.
39. Vasak C, Busenlechner D, Schwarze UY, Leitner HF, Munoz Guzon F, Hefti T, et al. Early bone apposition to hydrophilic and hydrophobic titanium implant surfaces: a histologic and histomorphometric study in minipigs. *Clin Oral Implants Res.* 2014;25(12):1378–85. <https://doi.org/10.1111/cir.12277>.
40. Bosshardt DD, Salvi GE, Huynh-Ba G, Ivanovski S, Donos N, Lang NP. The role of bone debris in early healing adjacent to hydrophilic and hydrophobic implant surfaces in man. *Clin Oral Implants Res.* 2011;22(4):357–64. <https://doi.org/10.1111/j.1600-0501.2010.02107>.
41. Kim S, Lee S, Cho I, Kim S, Kim T. Rotational resistance of surface-treated mini-implants. *Angle Orthod.* 2009;79(5):899–907. <https://doi.org/10.2319/090608-466.1>.

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III

**Differential modulation of osteoclastogenesis by macrogeometrically designed
hydrophobic and chemically modified hydrophilic titanium surfaces**

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Abstract

Objective: The aim of this study was to evaluate the influence of implant macrodesign and surface hydrophilicity on OC differentiation, activation, and survival *in vitro*.

Materials and Methods: Titanium disks were produced with a sandblasted, dual acid-etched surface, with or without additional chemical modification for increasing hydrophilicity (SAE-HD and SAE, respectively) and different macrodesign comprising of trapezoidal (HLX) or triangular threads (TMX). Four groups were included in the study: HLX/SAE-HD, HLX/SAE, TMX/SAE-HD, and TMX/SAE. RAW264.7 macrophages were seeded on the disks, differentiated to OC by RANKL treatment and cultured for 5 days. OC differentiated on polystyrene plates were used as positive controls [CCPC (+)]. OC differentiation and viability were assessed by enzymatic TRAP activity and MTT assays. Expression levels of various OC-related genes were measured at the mRNA level by qPCR.

Results: HLX/SAE-HD, TMX/SAE-HD, and HLX/SAE significantly suppressed OC differentiation when compared to CCPC (+). Cell viability was significantly increased in TMX/SAE and reduced in HLX/SAE-HD ($P < 0.001$). In addition, expression of IL-6 and TNF was upregulated in TMX/SAE-HD compared to CCPC (+) ($P < 0.05$ and $P < 0.01$, respectively).

Conclusion: Hydrophilic surfaces negatively modulate macrophage/osteoclast viability. Specifically, SAE-HD with double triangular threads increase the cellular pro-inflammatory status, while surface hydrophilicity and macrodesign do not seem to have a distinct impact on OC differentiation and activation.

Keywords: Dental implants, Hydrophilicity, Macrogeometry, Osseointegration, Osteoclasts, Gene expression

Introduction

Peri-implant bone healing follows a thoroughly organized and sequential tissue repair process, primarily dependent on cellular cross-talk among macrophages (M ϕ), osteoclasts (OCs), mesenchymal stem cells (MSCs) and osteoblasts (OBs), which finely couples the activities of bone resorption and new bone formation¹. During primary interfacial bone remodeling, activated osteoclasts absorb the native bone in direct contact with the implant to create favorable conditions for uneventful bone matrix synthesis and mineralization², resulting in transient implant stability drop after few weeks of implantation³. This event is especially critical when surgical instrumentation and placement of an implant generates interfacial stress and frictional force (*i.e.*, insertion torque) beyond the yield strain of the hard tissue⁴. This phenomenon results in ischemic bone necrosis due to micro-fractures and overcompression⁵, occurring mainly in the cortical bone and in the first implant threads, triggering bone remodeling and delaying the healing process^{6,7}.

Advances on oral implant technology, specifically modifications on macrodesign (*e.g.*, thread design), surface topography (*e.g.*, moderate roughness) and wettability (*e.g.*, hydrophilicity) have resulted in faster and superior quality of osseointegration, *i.e.*, bone-to-implant contact (%BIC), by modulating host-to-implant interactions^{8–10}. Specifically, thread design has an impact on implant primary stability and stress distribution at the bone-implant interface¹¹ and thereby also influences the amount/rate of osseointegration. For example, a trapezoidal and triangular thread-shaped design (standard V-thread) yields lower biomechanical stresses to the surrounding bone¹², which metabolism allows normal bone and prevents bone necrosis; while square and trapezoidal cutting chambers decrease the maximum micro motion and accelerate and enhance %BIC through an intramembranous-like healing pathway¹³.

In regards with surface topography, moderately-rough, sandblasted, large grid, acid-etched (SLA) surfaces show faster osseointegration compared to machined surfaces¹⁴, while additional treatment rendering the surfaces hydrophilic promotes further accelerated osseointegration. Hydrophilic surface treatment influences the early healing process by upregulating the expression of angiogenic factors and anti-inflammatory mediators, and downregulating the expression of pro-inflammatory cytokines, thereby directing osteogenic differentiation and maturation of MSCs (*i.e.*, contact osteogenesis)¹⁵.

More recently, evidence suggests that implant surface properties activate several components of the innate immune response following implant placement^{16,17}, which further modulate osteoclastogenesis in a surface dependent manner^{18,19}. Nevertheless, there is limited knowledge regarding the possible combined modulatory impact of implant macrogeometry and surface hydrophilicity on osteoclastogenesis. Thus, the present *in vitro* study aimed to evaluate the influence of implant thread design and surface hydrophilicity on OC differentiation, activation, and survival.

Materials and methods

Titanium disks

Disks (12 mm x 4 mm, Ø x L; Neodent®, Curitiba - PR, Brazil) were of commercially pure titanium (CpTi) grade IV, exhibiting 2 different macrodesign, marketed as:

- (1) Titamax® (TMX): double triangular threads (V-shaped) with similar grooves of 0.37 mm (Fig 1a).
- (2) Helix® (HLX): dynamic progressive thread geometry with double threads varying between square and trapezoidal design to triangular threads with cutting chambers exhibiting grooves of varied dimensions finishing in 0.55 mm (Fig 1b).

Further, each disk presented similar moderately rough microtopography, generated by means of sandblasting with 1.4 – 1.8 µm abrasive particles and dual acid-etching with hydrochloric (HCl) and sulfuric acid (H₂SO₄), but with varying surface wettability²⁰:

- (1) Acqua™ (SAE-HD): additional proprietary technology treatment, including microwave treatment under nitrogen protection to prevent air exposure, and insertion in 0.9% isotonic saline (NaCl) solution resulting in significant hydrophilicity (contact angle < 5°).
- (2) NeoPoros®: no further chemical modification (contact angle > 90°).

Thus, there were 4 experimental groups:

- (1) HLX/SAE-HD – progressive thread geometry with hydrophilic surface.
- (2) HLX/SAE – progressive thread geometry hydrophobic surface.
- (3) TMX/SAE-HD – triangular threads with hydrophilic surface.
- (4) TMX/SAE – triangular threads with hydrophobic surface.

All disks were initially sterilized by gamma (γ)-irradiation according to ISO 11137 (ISO, 2013) prior to processing. The disks were stored in sealed glass tubes to prevent hydrocarbon contamination and shipped under sterile conditions.

Mouse Mo/M ϕ cell line (RAW264.7) culture and differentiation to OC on titanium disks

RAW264.7, a murine macrophage-like cell line (ATCC® TIB-71™, LGC Standards GmbH, Wesel, Germany), was cultured at a density of 2×10^6 cells/ml in DMEM supplemented with 10% fetal bovine serum (FBS) and Penicillin-Streptomycin 10,000 $\mu\text{g/ml}$ (Gibco®, Gaithersburg, MD, USA) at 37°C, 5% CO₂ and 95% relative humidity atmosphere. To differentiate RAW264.7 to osteoclasts, cells were seeded on the experimental disks (n=3) placed in a 24-well plate at an initial population density of 3×10^5 /ml/well, subsequently treated with 100 ng/ml RANKL (PeproTech®, Rocky Hill, NJ, USA) and cultured for 5 days, in triplicate. RANKL-containing medium was replaced after 3 days. Osteoclasts differentiated on polystyrene, *i.e.*, cells seeded directly on polystyrene-surface wells and cultured for 5 days in the presence of RANKL, were used as positive control [CCPC (+)]. Cells cultured in the same fashion for only 12 h were applied as negative control [CCPC (-)]. The medium was removed after 5 days and cells were processed for further analysis.

Specific enzymatic Tartrate-Resistant Acid Phosphatase (TRAP) activity

TRAP activity was measured using the Acid Phosphatase Colorimetric Assay Kit (Abcam®, Cambridge, UK), according to the manufacturer's instructions. Readout of optical density at 405 nm was performed with a spectrophotometer. The final concentration of acid phosphatase (AP) was normalized to cell viability.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay

Cell viability of RAW-OC were assessed using the Vybrant® MTT Cell Proliferation Colorimetric Assay Kit (Thermo Fisher Scientific®, Waltham, MA, USA). The assay positive control group comprised cells treated with RANKL for 4 days and, additionally, stimulated with 100 ng/ml lipopolysaccharide (LPS) for 24 h (CCPC-LPS). The absorbance levels of each well were read at 570 nm using a spectrophotometer.

Reverse transcriptase real-time quantitative polymerase chain reaction (RT-qPCR): 2-step qPCR sample preparation

The RT-qPCR method was performed to measure the mRNA levels of genes related to osteoclastogenesis, OC activity and survival after 5 days of differentiation. The assay positive control group comprised cells treated with RANKL for 4 days and, additionally, stimulated with 100 ng/ml lipopolysaccharide (LPS) for 24 h (CCPC-LPS) to secrete inflammatory cytokines.

mRNA extraction and purification were performed applying the RNeasy Mini Kit (Qiagen®, Hilden, Germany). After 5 days of culture, 1×10^7 cells were mechanically harvested, lysed, and the cells lysate was processed according to the manufacturer's instructions. Total RNA fraction²¹ was quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific®, Waltham, MA, USA).

Reverse transcription for relative quantification of mRNA gene expression was performed using iScript™ cDNA Synthesis Kit (Bio-Rad®, Hercules, CA, USA). Subsequently, the cDNA served as template for amplification and relative quantification of selected genes by means of real-time quantitative polymerase chain reaction (qPCR). TaqMan® Gene Expression Assays (Applied Biosystems®, Foster City, CA, USA) and SsoAdvanced™ Universal Probes Supermix (Bio-Rad®, Hercules, CA, USA) were used to run the PCR reactions, according to the manufacturer's instructions. The gene expression levels of TRAP1, Cathepsin K (CTSK), Matrix Metalloproteinase (MMP)-9, Calcitonin Receptor (CALCR), Arginase 1 (ARG1), Interleukin (IL)-6, Tumor Necrosis Factor (TNF)- α and Bcl-2-associated X protein (BAX) were investigated. In order to correct interplate variations, an inter-run calibration sample (IPC; Interplate Calibrator FAM Probe, TATAA Biocenter AB, Göteborg, Sweden) was used in triplicate in every run and integrated in the qPCR data analysis workflow. The qPCR reaction was monitored with CFX Connect 96™ Real-Time System (Bio-Rad®, Hercules, CA, USA). The gene expression was quantified and fold regulation values were determined by normalizing cycle values (C_t) to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and beta-actin (ACTB; ΔC_t) and again to the positive control [CCPC (+)] C_t ($\Delta \Delta C_t$).

Qualitative and quantitative image analysis of osteoclast proliferation, adhesion and morphology

Scanning Electron Microscopy (SEM) analysis

Following 5 days of differentiation, cells were fixed in Sørensen phosphate-buffered glutaraldehyde solution (4%, 0.1 M, pH 7.4), dehydrated and, after critical point drying (BAL-TEC CPD 030, BalTec Group, Pfäffikon, Switzerland), the disks were sputter-coated with gold/palladium (Polaron SC7640, Quorum Technologies Ltd, Kent, UK). Images at different magnifications (10x, 100x, 500x, 1500x and 3000x) were obtained under a SEM microscope (JSM-5600LV, JEOL®, Peabody, MA, USA). The cellular morphology, adhesion and proliferation were analyzed in a region of interest (ROI) equivalent to the thread pitch of each macrogeometry (grooves of 0.37 mm; Fig 2).

Confocal laser scanning microscopy (CLSM) analysis

Following fixation and permeabilization of the cells (Image-iT™, Invitrogen®, Carlsbad, CA, USA), the actin cytoskeleton and cell nuclei were stained with phalloidin (0.1 µM/well; Alexa Fluor™ 488) and DAPI staining solutions (300 nm/well; FluoroPure™), respectively, according to the manufacturer's instructions (Invitrogen®, Carlsbad, CA, USA). Subsequently, disks were mounted in a small plexi and permanent mountant (ProLong™ Gold Antifade Mountant). Confocal microscope images (TCS SP8 DLS™, Leica®, Wetzlar, Germany) were obtained with optical section separation (z-stack) of a ROI equivalent to the thread pitch of each macrogeometry (grooves of 0.37 mm), comprising a length and voxel depth of 0.60 x 0.60 x 0.37 mm (X x Y x Z) at 25x magnification (Fig 2). Images were processed and used for 3D assessment of osteoclast size (µm²). 63x magnification (water immersion) micrographs were used for measurements of actin ring size (µm) and analysis of cell adhesion and morphology. Images were analyzed using ImageJ 2.0 software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

At least three independent experiments were performed for each analysis. Quantitative data were expressed as mean ± standard deviation (s.d.) and median. Initially, data were submitted to normality test (Shapiro-Wilk) and equal variance test (Levene). General linear statistical models were then applied for significant differences. MTT optical density and TRAP activity values were compared by one-way analysis of variance (ANOVA), followed by Turkey's *post hoc* test for multiple comparisons with the value of statistical significance set at the 0.001 level. For the gene expression analysis, the aforementioned test was performed for comparison of TRAP1 mRNA levels, followed by Dunn's *post hoc* test for comparisons with the positive control group ($P < 0.05$). Due to the asymmetrical distribution of data for gene expression levels

of CTSK, MMP9, CALCR, ARG1, IL6, TNF and BAX, cell size and actin ring size, a Kruskal-Wallis non-parametric ANOVA was used, followed by Dunn's *post hoc* test for comparisons with CCPC (+), and significance level was set at $P < 0.05$ and $P < 0.001$, respectively. The IBM SPSS Statistics software was used (IBM Company Inc., Chicago, IL, USA).

Results

Osteoclast differentiation and cellular viability

Following 5 days of RANKL-induced osteoclast differentiation, hydrophilic surfaces (HLX/SAE-HD and TMX/SAE-HD) significantly suppressed osteoclast differentiation ($P < 0.01$) when compared to the positive control group [CCPC (+)] (Fig 3a). Similarly, HLX/SAE resulted in significantly reduced TRAP activity ($P < 0.01$); while TMX/SAE group did not have any influence on the modulation of OC differentiation when compared to CCPC (+) ($P > 0.05$). CCPC (-) exhibited the minimum enzymatic activity compared to CCPC (+), TMX/SAE and HLX/SAE-HD ($P < 0.001$), HLX/SAE ($P < 0.01$) and TMX/SAE-HD ($P < 0.05$).

The results of the MTT assay showed significantly different absorbance levels among the experimental groups ($P < 0.001$, Fig 3b); specifically, increased viability in TMX/SAE disks and reduced viability in HLX/SAE-HD disks were observed. LPS induced OC activation and proliferation ($P = 0.001$) when compared to CCPC (+) and, therefore, used as control for gene expression of cytokine levels, being a stimulus that induces cytokine production.

OC-specific gene expression

TRAP1, CTSK, MMP9, CALCR, ARG1, IL6, TNF and BAX genes were expressed in all experimental and control groups. The following osteoclastogenesis and cell survival related genes did not show any statistically significant differences compared to CCPC (+): TRAP1, CTSK, MMP-9, CALCR, ARG1 and BAX ($P > 0.05$; Fig 4a-e, h). The only significant difference was in expression levels of genes associated with osteoclast-related inflammatory cytokines, when compared to the positive control group. Specifically, expression of IL-6 and TNF was more than 6-fold and 19-fold upregulated in TMX/SAE-HD, respectively ($P < 0.05$ and $P < 0.01$; Fig 4f-g). The mean C_t value of inter-run calibration replicates was 17.29 ± 0.66 cycles and their standard deviation in each run was less than 0.15 cycles (recommended s.d. < 0.3 cycles).

Qualitative image analysis of osteoclast morphology and adhesion

The first series of qualitative image assessment of RAW-OC differentiation, adhesion and morphological structures on different substrates investigated cells immunostained for F-actin (cytoskeleton) and DNA (cell nuclei). Magnified representative confocal micrographs demonstrate distinctive adhesion structures formed by multinucleated osteoclast-like cells and OCs on the experimental groups. Cells cultured on all substrates formed extensive podosomes, actin-derived structures associated with integrin-mediated cell adhesion. Single podosomes as well as actin rings were mainly distributed along the cellular cytoplasm of SAE-HD groups (Fig 5a and 6a), whereas predominantly larger but not necessarily more actin rings were formed on SAE surfaces (Fig 5g and 6g).

SEM images reveal cellular behavior in terms of adhesion and proliferation (Fig 5 and 6, b-f and j-l). RAW-OC cells differentiated on hydrophilic surfaces were found in randomly distributed small clusters on top of the thread tips and flanks, while compact cell assembly were identified predominantly in the valley, regardless of the macrogeometry (Fig 5 and 6, b-f). Relative to hydrophobic surfaces, strictly aggregated into clusters of OCs were identified along the entire pitch area, mainly in the valleys of both thread designs (Fig 5 and 6, h-l). The last phenomenon occurred particularly on the TMX group presenting triangular thread-shaped design, both on SAE-HD and SAE surfaces (Fig 6c and 6i). In turn, SEM analysis revealed similar tightened adhesion structures and filopodia projections of osteoclasts grown on both SAE-HD and SAE surfaces, but osteoclasts on SAE-HD surfaces presented with more elongated cell body and extended arms in comparison with SAE; macrogeometry did not appear to have any influence (Fig 5 and 6, e-f and k-l).

Quantitative image analysis of cell morphology and adhesion

Osteoclasts differentiated on HLX/SAE and TMX/SAE revealed the greatest range in size compared to HLX/SAE-HD, TMX/SAE-HD and CCPC (+) (Fig 7a). Significant differences were detected at the level of 99.9% for both hydrophobic disks in comparison with the control group and hydrophilic surfaces.

For the purpose of analyzing actin ring size of differentiated osteoclasts, only closed actin rings with a well-defined outline were included in the analysis, determined as the maximal width quantified in μm^2 . TMX/SAE group presented osteoclasts with the highest range in actin ring size (8.50 - 16.86 μm ; median 9.75 μm), however no statistically significant differences

were detected when compared to either the positive control group or the remaining experimental groups ($P \geq 0.05$; Fig 7b). Yet there was a tendency for the hydrophobic surfaces to present cells with larger actin rings than those presenting hydrophilicity. The smallest actin ring was measured on CCPC (+) group (2.77 – 4.10 μm ; median 3.4), followed by TMX/SAE-HD and HLX/SAE-HD (3.78 – 5.40 μm and 4.26 – 7.55 μm , respectively).

Discussion

The aim of the present study was to investigate the influence of thread design and surface hydrophilicity on OC differentiation, activation, and survival in a murine macrophage-like cell line model; the hydrophilic (SAE-HD) and hydrophobic (SAE) surfaces investigated herein present similar surface topography. Recently, Wennerberg et al. (2013)²³ validated the structural formation of nanoparticles on SLActive surfaces manufactured in a similar way as to SAE-HD herein, exhibiting physical characteristics similar to the cellular microenvironment.

Results obtained in the current study show that implant surface hydrophilicity negatively modulate osteoclast viability in the model of RANKL-induced osteoclastogenesis *in vitro*. Qualitative and quantitative assessment of cell morphology and adhesion revealed fundamental differences in a substrate-dependent manner. Integrin-based podosomes and actin rings, characterized in pre-mature and mature osteoclasts, respectively^{24,25}, were identified on all substrates. The total number of attached Mo/M ϕ and OCs on hydrophilic surfaces was decreased compared to hydrophobic surfaces. The latter was shown to facilitate cellular adhesion to the surface, which may have contributed to higher cellular viability. Correspondingly, when presenting hydrophilicity, moderately rough surfaces were shown to decrease attachment of monocytes and negatively modulate osteoclast differentiation in an osteoclastogenesis-induced model of murine bone-marrow derived macrophages (BMMs)¹⁸.

Recent *in vitro* studies have shown that topographical modifications of biomaterials affect the assembly of the sealing zone (SZ) and resorption apparatus (RA). Particularly, surface roughness at the micro and nanoscale level was shown to limit SZ expansion throughout ridge-like barriers and to interfere with RA formation^{26,27}. These observations support a limited and short-term lasting formation of actin rings on structured micro to nano-roughened substrates. Conversely, some studies support that osteoclastogenic differentiation and activation is equally increased by rough ($R_a > 2 \mu\text{m}$) and moderately-rough surfaces ($R_a = 1\text{--}2 \mu\text{m}$) as it occurs on

bone, while these mechanisms are considerably decreased on smooth substrates displaying nearly absent actin rings²².

In spite of differences in cellular attachment and viability, the present findings confirm that enzymatic TRAP activity of cells grown and differentiated on HLX/SAE-HD, HLX/SAE, and TMX/SAE-HD surfaces was significantly lower than on TMX/SAE surfaces and CCPC (+). However, no significant differences were detected at the mRNA level of gene expression encoding this enzyme. Although increasing synthesis of this acid phosphatase is an indicator of osteoclast formation and activity, it is also highly expressed in fused and activated macrophages in pathological states, playing a critical role in innate immune response²⁸.

Considering that translation and protein abundance depend on cytoskeleton rearrangement, the main reason behind this finding is the presence of biochemical signals on bone, but not on biomaterials. Consistently, Brinkmann et al. (2012)²² reported significant higher TRAP activity on bone compared to those grown on Ti surfaces. Hence, lack of significant differences at TRAP-related mRNA levels of gene expression on Ti disks may be explained by the fact that functional membrane structures, such as SZ, RA, and functional secretory domain (FSD), are not entirely generated by mature OCs when cultured *in vitro*, but only when in contact with osseous matrix (*i.e.*, phenotypic change of gene expression by environmental influence)²⁹. These changes in the cellular phenotype may result into disparities between gene expression and post-translational modification of protein levels³⁰. Furthermore, the correlation between mRNA and protein depends on other biological factors, such as cell cycle and its maturational stage, that may influence transcription levels, mRNA stability, translational rate, and protein turnover^{31,32}.

During osteoclast activity, cell-matrix interactions occur including release of hydrogen ions (vacuolar-type H⁺-ATPase) in order to facilitate bone demineralization and organic matrix exposure through secretion of lysosomal proteolytic enzymes into the resorption lacunae³³. For instance, MMP-9 and CTSK result in respective mineral and organic bone matrix (type I collagen) cleavage and degradation³³. Osteoclast activity-related gene expression revealed no significant effect of surface hydrophilicity and macrogeometry on bone resorption activity (CTSK, MMP-9 and CALCR), its negative regulator (ARG-1) nor on the expression of the pro-apoptotic gene BAX. Although there were no significant differences in the gene expression of

CALCR gene, its upregulation in the TMX/SAE-HD and TMX/SAE groups may imply stimulation of a more mature OC phenotype compared to the positive control group.

Gene expression levels of IL-6 and TNF- α were significantly upregulated in TMX/SAE-HD. This event might suggest a relationship between a low rate of osteoclast differentiation accompanied by cytokine production and promotion of a pro-inflammatory microenvironment. However, in previous studies, hydrophilic surfaces were shown to activate the highest production of anti-inflammatory factors and down-regulate the expression of key pro-inflammatory cytokines (*e.g.* IL-1 β , IL-6 and TNF- α) by osteoblasts (OB)³⁴ and macrophage-like cells¹⁶. Comparatively, the positive control group (cells differentiated on polystyrene-surface wells) revealed high level of ARG1 gene expression, suggesting prevalence of M ϕ 2 instead OC differentiation in the absence of the experimental biomaterial³⁵.

Although IL-6 and TNF- α are suggested to have an important impact on modulation of osteoclastogenesis^{36,37}, its mechanism of action during osseointegration has not yet been elucidated. Nevertheless, it is valid to acknowledge that the present methodology applying RANKL-induced osteoclastogenesis with the absence of a co-culture system limits further interpretation on the potential regulation of the pro-inflammatory status of macrophages by different surface treatments.

To date, this is the first study to evaluate osteoclast responses on hydrophilic substrates presenting different macrogeometry. Murine macrophage RAW264.7 cells have been extensively utilized in *in vitro* studies due to their expression of high levels of RANK and capacity to differentiate into OCs by treatment with RANKL³⁸. They are considered superior over the use of BMMs because of their purity, sensitivity to differentiation and prompt maturation, close correlation in gene expression and signaling, being functionally compared to primary isolated monocytes/macrophages³⁰. Nevertheless, results obtained herein shall be confirmed employing isolated murine/human BMMs in order to discriminate potential osteoclastogenesis-related processes against reported differences between immortalized macrophage cell line and primary macrophage-lineage cells (*e.g.* apoptosis/survival pathways and possible change in phenotype of subcultures)³⁹. Further implications considering prospective animal studies and clinical outcomes grounded on laboratory-based evaluations should be entirely taken as assumptions to be validated⁴⁰.

Conclusions

Double triangular threads together with chemically-modified hydrophilic surfaces appear to negatively modulate macrophage/osteoclast viability and increase their pro-inflammatory status in a model of RANKL-induced osteoclastogenesis. Surface hydrophilicity and macrodesign does not seem to have a distinct impact on osteoclast differentiation and activation.

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Conflict of interest

Rainde N. R. de Jesus received the experimental titanium disks from Neodent® (Curitiba-PR, Brazil; process no. PAP 0303-16) to support this study, as part of her doctoral research. None of the co-authors have any other conflict of interest in regards to this study.

References

1. Davies JE. Understanding peri-implant endosseous healing. *J Dent Educ* 2003;67(8):932–49.
2. Terheyden H., Lang NP., Bierbaum S., Stadlinger B. Osseointegration--communication of cells. *Clin Oral Implants Res* 2012;23(10):1127–35.
3. Han J., Lulic M., Lang NP. Factors influencing resonance frequency analysis assessed by Osstell™ mentor during implant tissue integration: II. Implant surface modifications and implant diameter. *Clin Oral Implants Res* 2010.
4. Eom T-G., Kim H-W., Jeon G-R., Yun M-J., Huh J-B., Jeong C-M. Effects of Different Implant Osteotomy Preparation Sizes on Implant Stability and Bone Response in the Minipig Mandible. *Int J Oral Maxillofac Implants* 2016;31(5):997–1006.
5. Bashutski JD., D'Silva NJ., Wang HL. Implant compression necrosis: current understanding and case report. *J Periodontol* 2009;80(4):700–4.
6. Coelho PG., Marin C., Teixeira HS., Campos FE., Gomes JB., Guastaldi F., et al. Biomechanical evaluation of undersized drilling on implant biomechanical stability at early implantation times. *J Oral Maxillofac Surg* 2013.
7. Trisi P., Todisco M., Consolo U., Travaglini D. High versus low implant insertion torque: a histologic, histomorphometric, and biomechanical study in the sheep mandible. *Int J Oral Maxillofac Implants* 2011;26(4):837–49.

8. Lang NP., Salvi GE., Huynh-Ba G., Ivanovski S., Donos N., Bosshardt DD. Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. *Clin Oral Implants Res* 2011;22(4):349–56.
9. Mendonça G., Mendonça DBS., Aragao FJL., Cooper LF. Advancing dental implant surface technology—from micron-to nanotopography. *Biomaterials* 2008;29(28):3822–35.
10. Elias CN., Rocha FA., Nascimento AL., Coelho PG., Eliasa CN., Rocha FA., et al. Influence of implant shape, surface morphology, surgical technique and bone quality on the primary stability of dental implants. *J Mech Behav Biomed Mater* 2012;16:169–80.
11. Chowdhary R., Halldin A., Jimbo R., Wennerberg A. Evaluation of stress pattern generated through various thread designs of dental implants loaded in a condition of immediately after placement and on osseointegration—an FEA study. *Implant Dent* 2013;22(1):91–6.
12. Lima de Andrade C., Carvalho MA., Bordin D., da Silva WJ., Del Bel Cury AA., Sotomaior BS. Biomechanical Behavior of the Dental Implant Macrodesign. *Int J Oral Maxillofac Implants* 2017;32(2):264–70.
13. Coelho PG., Jimbo R. Osseointegration of metallic devices: Current trends based on implant hardware design. *Arch Biochem Biophys* 2014;561:99–108.
14. Buser D., Schenk RK., Steinemann S., Fiorellini JP., Fox CH., Stich H. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. *J Biomed Mater Res* 1991;25(7):889–902.
15. Shanbhag S., Shanbhag V., Stavropoulos A. Genomic analyses of early peri-implant bone healing in humans: a systematic review. *Int J Implant Dent* 2015;1(1):5.
16. Hotchkiss KM., Ayad NB., Hyzy SL., Boyan BD., Olivares-Navarrete R. Dental implant surface chemistry and energy alter macrophage activation in vitro. *Clin Oral Implants Res* 2017;28(4):414–23.
17. Trindade R., Albrektsson T., Galli S., Prgomet Z., Tengvall P., Wennerberg A. Osseointegration and foreign body reaction: Titanium implants activate the immune system and suppress bone resorption during the first 4 weeks after implantation. *Clin Implant Dent Relat Res* 2018;20(1):82–91.
18. Bang SM., Moon HJ., Kwon YD., Yoo JY., Pae A., Kwon IK. Osteoblastic and osteoclastic differentiation on SLA and hydrophilic modified SLA titanium surfaces. *Clin Oral Implants Res* 2014;25(7):831–7.
19. Lotz EM., Berger MB., Schwartz Z., Boyan BD. Regulation of osteoclasts by osteoblast lineage cells depends on titanium implant surface properties. *Acta Biomater* 2018.
20. Sartoretto SC., Alves ATNN., Resende RFB., Calasans-Maia J., Granjeiro JM., Calasans-

- Maia MD. Early osseointegration driven by the surface chemistry and wettability of dental implants. *J Appl Oral Sci* 2015;23(3):279–87.
21. Solnestam BW., Stranneheim H., Hällman J., Käller M., Lundberg E., Lundeberg J., et al. Comparison of total and cytoplasmic mRNA reveals global regulation by nuclear retention and miRNAs. *BMC Genomics* 2012;13:574.
 22. Brinkmann J., Hefti T., Schlottig F., Spencer ND., Hall H. Response of osteoclasts to titanium surfaces with increasing surface roughness: an in vitro study. *Biointerphases* 2012;7(1–4):34.
 23. Wennerberg A., Svanborg LM., Berner S., Andersson M. Spontaneously formed nanostructures on titanium surfaces. *Clin Oral Implants Res* 2013;24(2):203–9.
 24. Georgess D., Machuca-Gayet I., Blangy A., Jurdic P. Podosome organization drives osteoclast-mediated bone resorption. *Cell Adhes Migr* 2014.
 25. Schachtner H., Calaminus SDJ., Thomas SG., Machesky LM. Podosomes in adhesion, migration, mechanosensing and matrix remodeling. *Cytoskeleton (Hoboken)* 2013;70(10):572–89.
 26. Geblinger D., Addadi L., Geiger B. Nano-topography sensing by osteoclasts. *J Cell Sci* 2010.
 27. Geblinger D., Zink C., Spencer ND., Addadi L., Geiger B. Effects of surface microtopography on the assembly of the osteoclast resorption apparatus. *J R Soc Interface* 2012.
 28. Ujiié Y., Todescan R., Davies JE. Peri-Implant Crestal Bone Loss: A Putative Mechanism. *Int J Dent* 2012.
 29. Saltel F., Bard F., Destaing O., Eichert D., Jurdic P. Apatite-mediated Actin Dynamics in Resorbing Osteoclasts. *Mol Biol Cell* 2004.
 30. Collin-Osdoby P., Osdoby P. RANKL-mediated osteoclast formation from murine RAW 264.7 cells. *Methods Mol Biol* 2012;816:187–202.
 31. De Sousa Abreu R., Penalva LO., Marcotte EM., Vogel C. Global signatures of protein and mRNA expression levels. *Mol Biosyst* 2009.
 32. Maier T., Güell M., Serrano L. Correlation of mRNA and protein in complex biological samples. *FEBS Lett* 2009.
 33. Vääriäniemi J., Halleen JM., Kaarlonen K., Ylipahkala H., Alatalo SL., Andersson G., et al. Intracellular machinery for matrix degradation in bone-resorbing osteoclasts. *J Bone Miner Res* 2004.
 34. Hyzy SL., Olivares-Navarrete R., Hutton DL., Tan C., Boyan BD., Schwartz Z.

- Microstructured titanium regulates interleukin production by osteoblasts, an effect modulated by exogenous BMP-2. *Acta Biomater* 2013;9(3):5821.
35. Yeon J-T., Choi S-W., Kim SH. Arginase 1 is a negative regulator of osteoclast differentiation. *Amino Acids* 2016;48(2):559–65.
 36. Wu Q., Zhou X., Huang D., Ji Y., Kang F. IL-6 enhances osteocyte-mediated osteoclastogenesis by promoting JAK2 and RANKL activity in vitro. *Cell Physiol Biochem* 2017.
 37. Bakker AD., Kulkarni RN., Klein-Nulend J., Lems WF. IL-6 alters osteocyte signaling toward osteoblasts but not osteoclasts. *J Dent Res* 2014.
 38. Hsu H., Lacey DL., Dunstan CR., Solovyev I., Colombero A., Timms E., et al. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. *Proc Natl Acad Sci U S A* 1999;96(7):3540–5.
 39. Berghaus LJ., Moore JN., Hurley DJ., Vandenplas ML., Fortes BP., Wolfert MA., et al. Innate immune responses of primary murine macrophage-lineage cells and RAW 264.7 cells to ligands of Toll-like receptors 2, 3, and 4. *Comp Immunol Microbiol Infect Dis* 2010.
 40. Coelho PG., Granjeiro JM., Romanos GE., Suzuki M., Silva NR., Cardaropoli G., et al. Basic research methods and current trends of dental implant surfaces. *J Biomed Mater Res B, Appl Biomater* 2009;88(2):579–96.

Figures

Fig 1 Schematic illustration and SEM micrographs of the threads' geometric profile [shape, width, depth, pitch, face length (mm) and helix angle] and surface microtopography of the experimental disks. **a** Titamax (TMX) contains double triangular threads (V-shaped) with similar grooves of 0.55 mm. **b** Helix (HLX) presents a dynamic progressive thread geometry with double threads varying between compressing trapezoidal design to self-tapping triangular threads with cutting chambers exhibiting grooves of varied dimensions finishing in 0.55 mm. **c-d** SEM micrographs of the surface microtopography following sandblasting and acid-etching treatment (1500x and 7000x magnification, respectively).

Fig 2 Schematic illustration of the region of interest (ROI) to qualitatively and quantitatively analyze osteoclast proliferation, adhesion and morphology under the SEM and CLSM. **a-c** HLX disks. **d-f** TMX disks. **a, d** Images of the ROI, equivalent to the thread pitch of each macrogeometry (grooves of 0.37 mm; blue area within the trapezoidal square), were obtained. **b, e** Three-dimensional view. **c, f** Two dimensional outlines of the ROI comprising a length and voxel depth of 0.60 x 0.60 x 0.37 mm (X x Y x Z) at 25x magnification.

Fig 3 RANKL-induced osteoclast differentiation and cellular viability following 5 days of culture on the negative control [CCPC (-)], positive control [CCPC (+)] groups, experimental Ti disks and assay control group (CCPC-LPS). **a** Specific enzymatic Tartrate-Resistant Acid Phosphatase (TRAP) activity assay. One unit of AP is the amount of enzyme causing the hydrolysis of one micromole of p-nitrophenyl phosphate (pNPP) to pNP per min (mU). Hydrophilic surfaces (HLX/SAE-HD and TMX/SAE-HD) significantly suppressed osteoclast differentiation ($P < 0.01$) when compared to the positive control group [CCPC (+)]. Similarly, HLX/SAE resulted in reduced TRAP activity ($P < 0.01$); while TMX/SAE group did not have any influence on the modulation of OC differentiation when compared to CCPC (+) ($P > 0.05$). CCPC (-) exhibited the minimum enzymatic activity compared to CCPC (+), TMX/SAE and HLX/SAE-HD ($P < 0.001$), HLX/SAE ($P < 0.01$) and TMX/SAE-HD ($P < 0.05$). **b** MTT assay. The results showed significantly different absorbance levels among all the experimental groups ($P < 0.001$); specifically, increased viability in TMX/SAE disks and reduced viability in HLX/SAE-HD disks were observed. Groups not sharing a letter are statistically significant at $\alpha = 0.05$ (*), 0.01 (**) and 0.001 (***).

Fig 2 mRNA gene expression levels related to osteoclastogenesis, and its negative regulation, OC activity and survival following 5 days of RANKL-induced osteoclast differentiation on the negative control [CCPC (-)], positive control [CCPC (+)] groups, experimental Ti disks and assay control group (CCPC-LPS). Results are expressed as relative normalized expression. Fold regulation values ($\Delta\Delta C_t$) were determined by normalizing cycle values (C_t) to GAPDH and ACTB (ΔC_t) and again to the positive control [CCPC (+)] ($\Delta\Delta C_t$). **a-e, h** Osteoclastogenesis and cell survival related genes did not show any statistically significant differences compared to CCPC (+): TRAP1, CTSK, MMP-9, CALCR, ARG1 and BAX ($P > 0.05$). **f-g** Expression of IL-6 and TNF was more than 6-fold and 19-fold upregulated in TMX/SAE-HD compared to CCPC (+) ($P < 0.05$ and $P < 0.01$, respectively). Data shown are the mean $\pm$ s.d. of three independent experiments including samples in triplicate and two technical replicates. Significance level was set at $\alpha = 0.05$ (*), $\alpha = 0.01$ (**) and $\alpha = 0.001$ (***).

HLX/SAE-HD

HLX/SAE

Fig 3 Qualitative image analysis of osteoclast proliferation, adhesion and morphology of RAW-OC cells following 5 days of RANKL-induced osteoclast differentiation on the experimental Ti disks. **a-f** HLX/SAE-HD. **g-l** HLX/SAE. **a, g** For confocal microscopy, cells were stained for actin cytoskeleton (*green*) and cell nuclei (*blue*). Confocal micrographs (63x magnification) demonstrate extensive podosomes and distinctive actin-derived structures associated with integrin-mediated cell adhesion formed by multinucleated osteoclast-like cells and OCs cultured on both disks. **b-f, h-l** The cellular morphology, adhesion and proliferation were analyzed under the SEM microscope in a region of interest (ROI) equivalent to the thread pitch of each macrogeometry (grooves of 0.37 mm). Micrographs (10x, 100x, 500x, 1500x and 3000x magnification) revealed RAW-OC cells differentiated on hydrophilic surfaces. **b-f** Cells were randomly distributed in small clusters on top of the thread tips and flanks, while compact cell assembly were identified predominantly in the valley, regardless of the macrogeometry. **h-l** Relative to hydrophobic surfaces, clusters of OCs were identified along the entire pitch area, mainly in the valleys of the HLX thread design. **e-f, k-l** SEM analysis at high magnification revealed similar tightened adhesion structures and filopodia projections of osteoclasts differentiated on both surface technologies.

Fig 6 Qualitative image analysis of osteoclast proliferation, adhesion and morphology of RAW-OC cells following 5 days of RANKL-induced osteoclast differentiation on the experimental Ti disks. **a-f** TMX/SAE-HD. **g-l** TMX/SAE. For confocal microscopy, cells were stained for actin cytoskeleton (*green*) and cell nuclei (*blue*). **a, g** Confocal micrographs (63x magnification) demonstrate extensive podosomes and distinctive actin-derived structures associated with integrin-mediated cell adhesion formed by multinucleated osteoclast-like cells and OCs cultured on both disks. **b-f, h-l** The cellular morphology, adhesion and proliferation were analyzed under the SEM microscope in a region of interest (ROI) equivalent to the thread pitch of each macrogeometry (grooves of 0.37 mm). Micrographs (10x, 100x, 500x, 1500x and 3000x magnification) revealed RAW-OC cells differentiated on hydrophilic surfaces. **b-f** Cells were randomly distributed in small clusters on top of the thread tips and flanks, while compact cell assembly were identified predominantly in the valley, regardless of the macrogeometry. **h-l** Relative to hydrophobic surfaces, clusters of OCs were identified along the entire pitch area, mainly in the valleys of the TMX thread design. **e-f, k-l** SEM analysis at high magnification revealed similar tightened adhesion structures and filopodia projections of osteoclasts differentiated on both surface technologies, without further influence of the macrogeometry when compared to HLX.

Fig 7 Quantitative image analysis of osteoclast proliferation and adhesion of RAW-OC cells following 5 days of RANKL-induced osteoclast differentiation on the experimental Ti disks. **a** Cell size (μm^2) of differentiated osteoclasts measured by means of confocal microscopy 3D processing of z-stack at 25x magnification. Osteoclasts differentiated on HLX/SAE and TMX/SAE revealed the greatest range in size compared to HLX/SAE-HD and TMX/SAE-HD. **b** Actin ring (mm) of differentiated osteoclasts measured by means of confocal microscopy at 63x magnification. TMX/SAE group presented osteoclasts with the highest range in actin ring size (8.5 - 16.86 μm ; median 9.75 μm), however no statistically significant differences were detected when compared to either the positive control group or the remaining experimental groups ($P \geq 0.05$). Box-plots of data represent the respective minima, first quartile, median, third quartile and maxima of cell size obtained from two independent experiments. Groups not sharing a letter are statistically significant at $\alpha = 0.001$ (***).

IV

Oversized surgical preparation of the implant site: systematic review and meta-analysis of preclinical studies

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Abstract

Objective: To answer the focused question: in animal models, do oral implants inserted in over dimensioned sites present greater biomechanical properties and histomorphometrical parameters of osseointegration compared to implants inserted in standard- and/or undersized-drilled implant sites?

Materials and Methods: Electronic databases were searched for controlled animal studies reporting on oversized, standard and/or undersized surgical preparation of the implant site. Relative implant-final drill discrepancy (IDD; mm) was calculated to categorize control and experimental groups: (1) control: standard and undersized drilling ($IDD \geq 0.2$ mm); (2) test OD: stress-free oversized drilling ($IDD = 0.0 - 0.1$ mm); and (3) test GAP: friction-free oversized drilling ($IDD \leq -0.1$ mm). The SYRCLE's Risk of Bias (RoB) tool was applied to assess the internal validity of the included articles. Random effects meta-analyses were performed for the outcomes of insertion and removal torque (N/cm; ITV and RTV, respectively), bone-to-implant contact (%BIC) and bone density (%BD) in short- (1-2 w), intermediate- (3-4 w) and long-term healing (≥ 5 w).

Results: Among the 943 records identified, 12 studies met the eligibility criteria, presenting with a low to unclear RoB. The meta-analysis indicated statistically significant benefits in favor of (a) control vs. test OD in short-term healing and extra-oral sites, with pooled estimates (WMD) of 25.35N/cm ITV and 26.19% BD; (b) control vs. test OD in long-term healing and intra-oral sites, with WMD of 11.69% BD; (c) control vs. test GAP in intermediate-term healing and extra-oral sites, with WMD of 3.03% BD; and (d) control vs. test GAP in long-term healing and extra-oral sites, with WMD of 5.57 N/cm RTV. In general, heterogeneity in the meta-analyses was low (I^2 0%) to moderate and high (I^2 53–88%).

Conclusion: Oversized surgical preparation of the implant site does not appear to provide any additional benefit compared to standard and undersized preparation, neither at the time of implant placement nor during the early healing period, yielding comparable results at the late post-operative period in different experimental animal models.

Keywords: dental implant, surgical protocol, oversized drilling, implant stability, bone-to-implant contact, animal models

Introduction

Primary implant stability (*i.e.*, mechanical interlocking between implant and bone as measured by maximum insertion torque values [ITV]) is recognized as a key factor to limit excessive micromotion (*i.e.*, minimal displacement relative to the implant site) and prevent osseointegration delay and/or impairment^{1,2}. Implant stability threshold is influenced by the interaction of multiple variables including surgical preparation of the implant site (*i.e.*, drilling technique), implant macrogeometry (body shape and thread design), recipient bone quality (structural composition and density) and quantity (volume and thickness), and occlusal stability^{3,4}. In the pursuit of high primary stability levels, excessive compression forces are employed with smaller-sized drills, specifically in cases of immediate loading where thin cortical and/or poorly dense trabecular bone is encountered (*e.g.*, atrophic posterior maxilla)^{5,6}.

Correspondingly, contemporary practice dictates that surgical drilling of the implant site is prepared slightly narrower than the implant diameter as per standard (press-fit condition where tapping of the host site is performed with the implant *per se* while the threads engage within the bone) and undersized drilling techniques (mismatch of the inner and outer implant diameters in relation to the drilled bone walls)⁷. During early stages of healing, regions of injured pristine bone in intimate contact to the implant hardware (*i.e.*, host bone-to-implant contact; h-BIC), initially responsible to ensure primary stability, undergo resorption to create adequate space over its critical threshold strain and support new bone deposition and maturation – characterizing an interfacial remodeling healing pathway⁸. Thus, implants presenting high levels of mechanical engagement (*i.e.*, high ITV) undergo transient implant biomechanical stability dip, significantly intensified by surgical trauma and microstructural stress beyond the yield strain of the bone⁹.

In this context, overcompression of the surrounding bone during implant placement produce micro-fractures and damage to the microcirculation with subsequent ischemic necrosis within the thread area, triggering non-solving inflammation and metabolic bone abnormalities^{10,11}. This process is termed as “necrosis dieback” and consists of coalescent bone remodeling sites occurring after 1 week following implant placement through activation of mechanosensitive cell-mediated response to microdamage, characterized by necrotic osteocytes and multiple bone remodeling units (BMUs)¹²⁻¹⁹. Consequently, excessive bone resorption occurs following the first weeks upon implant placement, reportedly leading to extensive decrease in total implant stability levels and eventual osseointegration delay in spite of high h-BIC.

Hence, a compressive strain release technique has been investigated by employing a larger final drill outer diameter (*i.e.*, oversized drilling) throughout a stepwise drilling instrumentation to achieve either similar dimension between the bone defect and the implant outer diameter or over dimensioned sites^{12,20}. Oversized drilling in interplay with implant macrogeometry creates closed healing chambers, namely void spaces, shaped in the inner thread region between the inner implant diameter and the surrounding native bone that allow blood clot stabilization immediately after surgical placement^{8,21}. The early healing kinetics in healing chambers are capable of positively modulating cell-mediated responses towards intramembranous-like ossification rather than interfacial bone remodeling¹⁵. As a result, its high bone appositional rate supports a rapidly and uneventful osseointegration onset of unloaded implants, increasing secondary stability already in the first week *in vivo* despite low ITV and h-BIC^{11,22}.

Nevertheless, the optimal level of primary stability to lessen the healing period and enhance osseointegration has been questioned¹⁴. The research to date is remarkably controversial regarding the correlation between high ITV and implant stability quotient (ISQ)^{6,23-26}. Due to the lack of corresponding data, the primary aim of the present study was to systematically assess the current evidence reporting on oversized surgical preparation of the implant site in terms of biomechanical properties and histomorphometrical parameters of osseointegration in experimental animal models. The secondary aim was to perform a meta-analysis of the estimate of efficacy of oversized *vs.* standard or undersized surgical preparation of the implant site.

Materials and Methods

Focused Question

This study follows the guidelines of the Preferred Reporting of Systematic Review and Meta-analyses (PRISMA) statement²⁷. Before commencement of the present systematic search, a comprehensive review protocol was established and successively approved by all authors. The focused ‘PICO’ (population, intervention, comparison, outcome) question²⁸ was: in preclinical *in vivo* and *ex vivo* experimental models, do oral implants inserted in over dimensioned sites present greater biomechanical properties and histomorphometrical parameters of osseointegration compared to implants inserted in standard- and/or undersized-drilled implant sockets?

Article Inclusion Criteria

Studies reporting original research comparing oversized implant drilling technique to regular and/or undersized implant drilling technique were included in the present systematic review according to the following inclusion criteria:

- (1) Randomized or non-randomized controlled preclinical *in vivo* studies.
- (2) Use of intra-oral or extra-oral experimental sites of skeletally small or large animals.
- (3) Presence of at least one control group (*i.e.*, standard and/or undersized drilling technique).
- (4) Studies published in English until July 2018.
- (5) Including one of the following analysis: micro-computed tomography (μ CT), radiographic assessment, histomorphometric and histologic analysis, or biomechanical test.

The following exclusion criteria were applied:

- (1) *Ex vivo* and/or *in vitro* studies.
- (2) Studies comparing different implant macrogeometries between test and control groups.
- (3) Studies evaluating oversized drilling applied only in the cortical layer (*i.e.*, profiling).
- (4) Animal studies reporting orthodontic implants (mini-screw implants), short implants (≤ 6 mm) and/or orthopaedic implants.
- (5) Studies evaluating regenerative techniques and simultaneously placement of implants using autogenous bone and/or biomaterial-based grafting in combination with barrier membranes.

No restrictions were applied regarding animal species, year of publication, sample size, implant system (macro- and microgeometry), surface technology, time of implant placement, and observation period.

Outcome Measures of Interest

The biologic outcomes, mainly reported as histomorphometrical parameters of osseointegration in preclinical *in vivo* research, were selected as primary outcome measures of interest: (1.1) percentage bone-to-implant contact (%BIC), expressed as the relative amount of bone growth onto the implant surface within the implant site or within a region of interest, and (1.2) percentage bone density (%BD), expressed as the relative amount of the total area of mineralized bone within the implant site or within a region of interest. The biomechanical outcomes, representing the mechanical resistance at the bone-implant interface, were selected as secondary outcome measures of interest: (2.1) insertion torque values (ITV; N/cm), measured as the maximum torque employed during implant placement (*i.e.*, biomechanical anchorage), (2.2) removal torque values (RTV; N/cm), as the maximum torque obtained in the

unscrewing process (*i.e.*, biomechanical resistance indicative parameter of the quality of implant osseointegration) and (2.3) implant stability quotient (%ISQ), considered as a measure of primary implant stability (*i.e.*, strength of implant anchorage in the bone).

Search Strategy, Screening Methods, and Study Selection

Electronic databases of MEDLINE (via PubMed), Web of Knowledge, Scopus and Cochrane Wiley Library were searched for relevant English-language literature with a platform-specific search strategy (Tables S1-S4) up to and including July 2018. The search strategy consisted of combinations of medical subject headings (MeSH) terms, keywords enclosed in both title and abstract for all databases, and Boolean operators. Additionally, bibliography of the selected studies and relevant review articles were manually searched for cross-references. A double-blind three-stage screening process was carried out by two independent reviewers (R.N.R.J. and A.S.) to assess relevant studies. At the first and second stages, screening of titles and abstracts of the search-identified studies were performed, respectively. Studies showing to assemble the eligibility criteria, or those presenting insufficient information qualified for inclusion, were selected for further assessment. The final selection of articles at the third stage screening-process was provided by a proof of reading of full texts of all eligible studies. Following each stage, a consensus was taken by R.N.R.J. and A.S. to discuss agreements and disagreements about inclusion or exclusion criteria of each study. In case of disagreement, a third co-author was designated as the mediator (D.Z.B.). Agreement between reviewers was described by interrater reliability (Cohen's kappa coefficient; κ)²⁹. When completing a comprehensive full-text assessment, further studies were reportedly excluded and respective explanations were given.

Data Extraction

One reviewer (R.N.R.J.) was entitled to extract the data of the studies in the final selection, followed by an appraisal by a second reviewer (A.S.). Descriptive summaries of respective biomechanical properties and histomorphometrical parameters of osseointegration were further entered into tables, including the authors' conclusions. Standard errors of means and confidence intervals, when reported, were converted to standard deviation values. In case of missing or misplaced data, the corresponding author was contacted via email. Mean $\pm$ standard deviation (s.d.) were finally reported. Significant differences between groups were accordingly identified by symbols. The relative dimensional mismatch between the outer implant diameter and the bone wall in the implantation bed (implant-last drill discrepancy; IDD, expressed in mm and

relative %) was calculated to classify control and experimental groups for meta-analyses and further comparisons. Due to large variation in the methodology of the eligible studies, studies were pooled based on homogeneity regarding 'PICO'. The dimension of oversized implant sites was classified into two levels, according to the most frequently reported IDD in the pertinent literature:

- (1) Control: press-fit/standard drilling technique ($IDD = 0.2 - 0.5 \text{ mm} \approx 3 - 15\%$) to undersized drilling technique ($IDD > 0.5 \text{ mm}/15\%$).
- (2) Test OD: oversized drilling technique ($IDD = 0.0 - 0.1 \text{ mm} \approx 0 - 2\%$) to achieve either nearly or exactly similar dimension between the bone wall and the implant outer diameter (stress-free).
- (3) Test GAP: oversized drilling technique ($IDD \leq -0.1 \text{ mm}/-3\%$) presenting a gap between the bone wall and the implant outer diameter (friction-free).

Quantitative biomechanical (ITV and RTV) and histomorphometric data (%BIC and %BD) were then extracted for meta-analysis. When studies included multiple standard and/or undersized drilling protocols, the one with the least IDD was included in the control group. Outcomes reported in different time points were pooled in three major periods of healing as reported in the literature as short- (1-2 w), intermediate- (3-4 w) and long-term healing ($\geq 5 \text{ w}$).

Risk of Bias (RoB) and Quality Assessment

The SYRCLE's RoB tool for animal studies, based on the Cochrane Collaboration RoB tool, was applied to assess and report the internal validity of the included articles. Risk of bias was assessed as 'high' (-), 'unclear' (?) or 'low' (+)³⁰ (Table S5).

Modified ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines were applied to assess and report the quality of the studies regarding relevant items³¹. A predefined grading system was applied to each of the 20 items to evaluate the reporting quality of the eligible studies³² (Table S6).

Meta-analysis

A meta-analysis was performed to compare biomechanical and biologic outcomes of oversized (experimental) and undersized/standard (control) implant drilling techniques applying biomechanical (ITV and RTV) and histomorphometric (%BIC and %BD) data [means $\pm$ s.d. and number of animals (n) per group] in small and large animal models. The DerSimonian and

Laird random effects model was applied using STATA Statistical Software (StataCorp LP, College Station, TX, USA) to determine if one approach has statistical superiority compared to the other. Since the study design for all studies included multiple interventions per animal (*i.e.*, ‘split-mouth’ design), a correlation coefficient (R-value of 0.50) was applied for between-treatment group measurements³³. Pooled estimates of treatment effect (weighted mean differences [WMD]; *i.e.*, mean of the test group minus mean of the control group divided by the pooled s.d. of both groups) and 95% CI were calculated. Statistical heterogeneity was assessed by graphic display and by determining the consistency of the WMD from the included studies in conjunction with 95% CI. The chi-square test was used to assess heterogeneity, which classification was established as low (25%), moderate (25%), and high (25%) degree³⁴. The test for homogeneity (I^2) was assumed to quantify the extent of heterogeneity before each analysis, whenever feasible. Forest plots were generated for analysis of data and significance level was set at $P < 0.05$.

Results

Study Selection

The PRISMA flow chart of the present systematic review are illustrated in Figure 2. The initial electronic search strategy resulted in hypothetically applicable 990 records at four selected databases. Following exclusion of duplicates and the first-stage screening of titles and abstracts, 20 full-text articles, which were assessed for eligibility according to the inclusion and exclusion criteria. Following the full-text assessment, 12 articles were included in the present systematic review (Table S7). The reasons for exclusion of 8 articles are reported in Table S8.

Characteristics of Included Studies

An overview all 12 studies regarding objectives, method of evaluation, animal model, implant system, implant material, surface treatment, implant shape and dimensions is shown in Table 1. Further characteristics of the study design were also assessed (Figure S1). The majority of the studies adopted dogs as the animal model (58%), followed by sheep (17%), rabbits (17%) and minipigs (8%). Regarding biomechanical analysis, 7 studies investigated the effects of oversized drilling technique on ITV (38%), RTV (24%) and %ISQ (38%) in both intra-oral (24%) and extra-oral sites (76%). In 10 studies, biologic parameters were investigated, including %BIC (100%) and %BD (82%) in intra-oral (40%) and extra-oral sites (60%).

Regarding implant systems, most of the studies applied moderately rough surfaces with or without additional chemical modification. Regarding implant macrogeometry, a total of 9 articles evaluated conical/tapered implants, while only 3 used cylindrical implants. Four studies evaluated implants with triangular-shaped threads, 5 applied a trapezoidal thread design, and 3 others analysed implants with a combination thereof (*i.e.*, dynamic progressive thread design). The dimensions of the implants ranged from 8 mm to 10 mm in length (L) and 3.4 mm to 4.5 mm in diameter (Ø). In total, 11 studies (92%) included SD as the control group, whereas only 5 studies (42%) evaluated UN in comparison to test OD and/or test GAP.

Risk of Bias and Quality Assessment

Overall, studies were of low to unclear risk of bias (Figure 3 and 4) and adequately provided information regarding title, abstract, introduction, study objectives, ethical statement, experimental design, baseline characteristics of experimental animals, surgical procedures, post-operative adverse events and relevance to human biology and translation (Table 2). All studies provided adequate information on animal characteristics at baseline and reported a detailed outcome assessment. However, only 8% of the studies reported on suitable random sequence generation and blinding of assessor. Information regarding sample size calculation and health status of the animals, including housing and husbandry, and implications of the 3Rs (replacement, refinement and reduction) was mostly insufficient or missing. Allocation concealment, random housing of the animals in the facility, blinding of caregivers and/or investigators, calibration of surgeons, and random outcome assessment were missing.

Descriptive Histology

Descriptive histology of implants inserted in oversized- compared to standard- and/or undersized-drilled sites analyzed herein was assessed in 10 studies, depicting different healing patterns among test and control groups (Table 4 and S10).

In general, oversized surgical preparation of the implant site was characterized by healing chambers filled with osteogenic tissue and extensive woven bone formation in the early healing period, indicating an intramembranous-type healing mode, in comparison to sites in absence of void spaces^{11,12,14-17,35,36}. None of the studies found micro-fractures in the peri-implant bone or ischemic necrosis following implant placement in oversized sites. At the late healing period, implants were well osseointegrated into comparable mineralized bone in healing chambers and in contact to the implant surface, regardless of the surgical protocol³⁵⁻³⁸.

In turn, standard and undersized drilling techniques resulted in collagen deformation and microcracks in the cortical bone¹¹, extensive areas of necrotic bone and substantial remodeling, especially in the primary thread engagement area around cortical bone^{12,15,18,35}. Only 1 study did not observe any negative impact of standard/undersized drilling in the healing aspect of cortical bone at the tissue level, in an extra-oral model of sheep (iliac crest).

Meta-analysis of pre-clinical *in vivo* studies

Descriptive summaries of respective biomechanical properties and histomorphometrical parameters of osseointegration of studies included in the meta-analysis were further entered into Table 3 and 4, respectively. Biomechanical data from 2 studies comparing test OD vs. control were pooled for the outcome of insertion torque in short-term healing, and from 2 studies comparing test GAP vs. control for the outcome of removal torque in long-term healing and in extra-oral sites (dogs and sheep; Figure 5). Histomorphometric data from 3 studies comparing test OD vs. control were pooled for the outcome of %BIC in intermediate- and long-term healing (Figure 6), and from 5 studies for the outcome of %BD in short-, intermediate- and long-term healing in intra-oral (dogs and miniature pigs) and extra-oral (sheep, dogs and rabbits) experimental sites (Figure 7). For detailed information on biomechanical and biologic outcomes of all 12 included studies, please refer to Table S9 and S10, respectively.

Overall, the meta-analysis revealed statistically significant effects in favour of:

- (a) control vs. test OD in short-term healing and extra-oral sites, with pooled estimates (WMD) of 25.35N/cm insertion torque (95% CI: 20.94, 29.75, $P = 0.000$; Figure 5a) and 26.19% BD (95% CI: 20.10, 32.27, $P = 0.000$; Figure 7a).
- (b) control vs. test OD in long-term healing and intra-oral sites, with WMD of 11.69% BD (95% CI: 6.98, 16.40, $P = 0.000$; Figure 7c).
- (c) control vs. test GAP in intermediate-term healing and extra-oral sites, with WMD of 3.03% BD (95% CI: 0.44, 5.63, $P = 0.02$; Figure 7d).
- (d) control vs. test GAP in long-term healing and extra-oral sites, with WMD of 5.57 N/cm removal torque (95% CI: 1.26, 9.88, $P = 0.000$; Figure 5b).

Overall, low ($I^2 = 0\%$) to moderate and high ($I^2 = 53\text{--}88\%$, respectively), was observed in the analyses as a measure of inconsistency of results across studies.

Discussion

The aim of this study was to systematically review the preclinical *in vivo* evidence for oversized surgical preparation of the implant site and to perform a meta-analysis of the estimate of efficacy of oversized vs. standard and/or undersized surgical protocols in terms of biomechanical properties and histomorphometrical parameters of osseointegration in experimental animal models. In regards with biomechanical outcomes, the meta-analysis showed that oversized surgical protocols (test OD [IDD = 0.0 – 0.1 mm] and test-GAP [IDD = ≤ -0.1 mm]) resulted in significantly decreased values of maximum insertion torque (ITV) and removal torque values (RTV), compared to standard surgical protocol (SD; IDD = 0.35 – 0.5 mm) evaluated in extra-oral sites of large animals. On the other hand, oversized surgical protocols prevented ischemic necrosis and extensive bone resorption at the bone-implant interface, both in the marginal cortical layer and in the trabeculae. Consequently, faster and increased rates of bone formation were reported to occur around implants placed in over dimensioned sites, characterized by primary osteons and highly vascularized tissue following 1 week up to 5 weeks of healing. Histomorphometrically, test OD resulted in overall reduced bone-to-implant contact (%BIC) in the early period of healing, while statistically similar values were revealed following 3 weeks of healing compared to SD (IDD = 0.2 – 0.55 mm). Additionally, test OD showed significantly reduced relative bone density (%BD) up to 2 weeks and, again, following 5 weeks of healing – with similar amounts between 3 and 4 weeks compared to SD (IDD = 0.2 – 0.55 mm). Meanwhile, during this period, test GAP resulted in reduced values compared to SD (IDD = 0.2 – 0.4 mm).

First, primary implant stability is relative to the degree of lateral bone compression as it corresponds to the mechanical resistance of the implant against micromotion immediately and early after surgical placement^{39,40}. Clinically, a minimum ITV of ≥ 30 N.cm has been proposed as the fundamental criteria for immediate implant loading^{41,42}. In a result, medium to high ISQ (56 – 82%)^{7,43} and tolerable micromotion threshold (50 – 150 μ m)⁴⁴ have been recognized as fundamental parameters for treatment success^{1,45}. Despite the ever-growing scientific evidence on the clinical significance of these parameters and their complexity, high ITV may not be translated into corresponding high primary stability, as previously mentioned²³.

Despite significantly decreased ITV and ISQ upon implant placement, studies show that over dimensioned sites (test OD and test GAP) result in substantial increase in implant stability over time, whereas standard- and undersized-drilled sites reveal a tendency to maintain stable values with minimal increase up to 6 weeks, in both intra- and extra-oral sites^{11,22}. Owing to a stepwise

drilling instrumentation and passive surgical installation, implants placed in overprepared sites do not exert frictional and compressive forces, resulting in low to nearly absent mechanical engagement of the threads within the bone^{12,15}. This situation is translated into low ITV (≤ 20 N.cm), particularly in low density bone (type IV). Comparably, implants installed in narrower sites yield high ITV when prepared with standard (≥ 30 N.cm) and undersized drilling techniques (≥ 50 N.cm), as a result of increased friction and compression of the surrounding bone during implant insertion, especially in thick cortical and dense trabecular bone. Notwithstanding, test GAP showed a smaller difference in secondary implant stability, as assessed by RTV, but statistically significantly lower compared to SD.

The biologic findings reflect the abovementioned fast achievement of secondary stability in oversized sites, while SD and UN undergo extensive bone remodeling prior to osteogenic tissue apposition, especially between 2 and 4 weeks. However, the presence of a gap between the implant and the bone wall appears to not necessarily outperform the amount of bone density observed in standard-drilled sites. Healing chambers filled with blood clot have been shown to modulate cellular responses during the early process of tissue repair by creating an osteoprotective microenvironment with a provisional matrix/scaffold that supports osteoconductivity of novel bioactive surfaces^{46,47}. Surface chemistry stimulates a constant exchange of fluid and ions that further induces biochemical binding, eliciting specific cellular response towards contact osteogenesis⁴⁸⁻⁵².

In this scenario, multidirectional bone growth occurs on the implant surface and from the surgically instrumented pristine bone walls towards the central region of the chamber throughout an intramembranous-like healing pathway^{11,20,53}. As healing chamber configurations do not cause “necrosis dieback”, void spaces are rapidly filled with woven bone presenting high degrees of vascularization and cellular composition already in the first week upon implant placement with subsequent maturation surrounding the perimeter of primary osteons up to 3 weeks^{11,12,14-17}. Similar microstructures have been previously found in healing chambers shaped around plateau implants as early as 2 and 5 weeks *in vivo* in association with multiple modelling sites, where the bone formation rate varied between 30 and 50 μm per day²⁰. Thus, oversized sites might contribute to strength bone mechanical properties throughout formation of a multidirectional harversian-like channel system, and thereby promptly adapting to functional overloading stress⁵⁴⁻⁵⁷.

Noteworthy is that implant body shape and thread design influence the degree of lateral mechanical compression and stress distribution at the bone-implant interface upon surgical placement as well as under axial implant loading⁵⁸. The level of peri-implant interfacial strain concentration affects bone metabolism, microstructural composition, and biomechanical resistance (e.g., stiffness). For instance, tapered implants are routinely indicated for the rehabilitation of challenging cases (e.g., posterior maxilla, immediate placement in fresh sites and/or immediate loading of single crowns) as they achieve better primary stability when compared to cylindrical implants⁵⁹. Concerning the effect of thread geometry (shape, depth, pitch and angle), cutting trapezoidal and triangular threads generate lower stress concentration in bone^{58,60}, whereas compressive square and trapezoidal chambers present significantly higher biomechanical fixation throughout the healing period⁶¹.

Recently, studies have suggested the benefit of hybrid healing pathway to enhance the performance of implants following placement in over dimensioned sites by integrating both interfacial remodeling and intramembranous-like healing modes^{18,35,38}. Specifically, implants with a dynamic progressive thread profile, engineered with various shapes and dimensions through the implant length, may contribute for attaining adequate mechanical resistance to micromotion, while preventing compressive strain in the highly dense crestal and trabecular bone. The rationale behind this association lies upon the fact that the tip of triangular self-tapping threads slightly engages into bone surrounding the implant apex, while square and trapezoidal threads in the central coronal portion of the implant form healing chambers due to compressive stress release. Fast woven bone formation and ossification compensate the potential loss of primary stability that will follow the events of interfacial remodeling in sites initially in direct contact to the implant surface, hastening osseointegration already in the early healing period⁶². Hence, this situation may prevent implant stability dip while contributing to a faster osseointegration^{60,63}. However, one should bear in mind that, depending on the status of host bone and loading condition, mechanical resistance against micromotion in over dimensioned sites might reach similar levels compared to those instrumented with standard and/or undersized drilling techniques during the early post-operative period.

Furthermore, the clinical effect of the interplay between implant macrogeometry and surgical technique, in terms of bone compression and microdamage, is directly dependent on bone density and loading condition, directly affecting marginal bone levels (MBL). In cases of thick poorly vascularized cortical and/or highly dense trabecular bone, surgical preparation of the

cortex is further enlarged with profiling (*i.e.*, countersink) in addition to tapping of the host walls. This technique prevents early marginal bone loss along with an increase in the rate of BIC and bone density in this region as time elapses, in contrast with undersized techniques¹¹. In contrast, overcompression of the cortical bone appears to have a deleterious effect to the peri-implant bone surrounding undersized sites in the absence of profiling under immediate functional loading⁶⁴. However, studies reporting on MBL under the influence of various surgical drilling protocols are limited and do not allow further assumptions. Likewise, the great variability in study design (*e.g.*, implant systems, animal models, absence of loading condition, etc.) and quality of methodological analysis of the reviewed articles may have an effect in the calculation of pooled estimates and interpretation should be performed with care.

While no animal species completely fulfil all the requirements of an ideal model to test biomaterials prior to clinical testing, an appreciation of their physiological and anatomical aspects (*e.g.*, bone metabolic rate, mineral density, and site dimensions) contribute to interpretation of significant data⁶⁵. Concerning desirable attributes of animal models to analyse potential effects of varied surgical techniques of the implantation bed, the mandible of large animals offers nearly similar bone composition and density compared to humans and, therefore, present a more equivalent bone remodeling pattern^{66,67}. However, potential deleterious effects might be worsened in a synergic manner owing to its thick cortical layer and highly dense trabecular bone. Nevertheless, intra-oral model of large animals further allows functional loading and, specifically to dogs, oral hygiene monitoring and infection prevention. In this context, rabbits show the least advantage. The limited application of various surgical locations is based on the fact that the quality of bone differs between anatomical sites and even within a single limb⁶⁸, from mesial to distal sites, substantially leading to distinct tissue regeneration and integration from that found intra-orally in humans⁶⁹. The most beneficial application of tibia as an implantation site lays on the fact that it offers better discriminating potential to evaluate osteoconductive properties of modified implant surfaces to enhance bone formation due to its large lumen, which is relatively poor in trabecular bone⁷⁰. Finally, better practices in performing animal science to evaluate dental implants must be implemented, including animal selection, study design, handling, among others, in order to improve the quality of reporting data and translational applications^{31,71}.

Conclusion

Oversized surgical preparation of the implant site appears to minimize marginal bone resorption characterized by fast rates of new bone formation in the healing chambers through an intramembranous-type healing mode. Within the limitations of this systematic review and meta-analysis, it can be concluded that, in terms of biomechanical properties and histomorphometrical parameters of osseointegration, oversized implant sites does not seem to provide any additional benefit compared to narrower implant sites, neither at the time of implant placement nor during the early healing period, yielding comparable results at the late post-operative period in different experimental animal models.

No conclusive remarks on the clinical implications of varied surgical protocols on osseointegration of different implant systems can be made due to limited magnitude of treatment effect size and heterogeneity of the observational studies. Further controlled basic and translational clinical research under the influence of functional loading is warranted to enhance clinical decision-making.

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Conflict of interest

None of the co-authors have any competing interest to declare.

References

1. Javed F, Ahmed HB, Crespi R, Romanos GE. Role of primary stability for successful osseointegration of dental implants: Factors of influence and evaluation. *Interv Med Appl Sci.* 2013;5(4):162-167.
2. Duyck J, Vandamme K, Geris L, et al. The influence of micro-motion on the tissue differentiation around immediately loaded cylindrical turned titanium implants. *Arch Oral Biol.* 2006;51(1):1-9.

3. Coelho PG, Jimbo R. Osseointegration of metallic devices: Current trends based on implant hardware design. *Archives of Biochemistry and Biophysics*. 2014;561:99-108.
4. Albrektsson T, Brånemark PI, Hansson HA, Lindstrom J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. *Acta Orthop Scand*. 1981;52(2):155-170.
5. Alghamdi H, Anand PS, Anil S. Undersized implant site preparation to enhance primary implant stability in poor bone density: a prospective clinical study. *J Oral Maxillofac Surg*. 2011;69(12):506.
6. Degidi M, Daprile G, Piattelli A. Influence of underpreparation on primary stability of implants inserted in poor quality bone sites: an in vitro study. *J Oral Maxillofac Surg*. 2015;73(6):1084-1088.
7. Friberg B, Sennerby L, Gröndahl K, Bergström C, Bäck T, Lekholm U. On cutting torque measurements during implant placement: a 3-year clinical prospective study. *Clin Implant Dent Relat Res*. 1999;1(2):75-83.
8. Berglundh T, Abrahamsson I, Lang NP, Lindhe J. De novo alveolar bone formation adjacent to endosseous implants: A model study in the dog. *Clin Oral Implants Res*. 2003;14(3):251-262.
9. Gomes JB, Campos FE, Marin C, et al. Implant biomechanical stability variation at early implantation times in vivo: an experimental study in dogs. *Int J Oral Maxillofac Implants*. 2013;28(3):128.
10. Bashutski JD, D'Silva NJ, Wang HL. Implant compression necrosis: current understanding and case report. *J Periodontol*. 2009;80(4):700-704.
11. Eom T, Kim H, Jeon G, Yun M, Huh J, Jeong C. Effects of Different Implant Osteotomy Preparation Sizes on Implant Stability and Bone Response in the Minipig Mandible. *Int J Oral Maxillofac Implants*. 2016;31(5):997-1006.
12. Campos FE, Gomes JB, Marin C, et al. Effect of drilling dimension on implant placement torque and early osseointegration stages: an experimental study in dogs. *J Oral Maxillofac Surg*. 2012;70(1):43.
13. Jimbo R, Tovar N, Anchieta RB, et al. The combined effects of undersized drilling and implant macrogeometry on bone healing around dental implants: an experimental study. *Int J Oral Maxillofac Surg*. 2014;43(10):1269-1275.
14. Campos FE, Jimbo R, Bonfante EA, et al. Are insertion torque and early osseointegration proportional? A histologic evaluation. *Clin Oral Implants Res*. 2015;26(11):1256-1260.

15. Baires-Campos F, Jimbo R, Bonfante EA, et al. Drilling dimension effects in early stages of osseointegration and implant stability in a canine model. *Med Oral Patol Oral Cir Bucal*. 2015;20(4):471.
16. Duyck J, Roesems R, Cardoso MV, Ogawa T, De VC, Vandamme K. Effect of insertion torque on titanium implant osseointegration: an animal experimental study. *Clin Oral Implants Res*. 2015;26(2):191-196.
17. Coelho PG, Marin C, Teixeira HS, et al. Biomechanical evaluation of undersized drilling on implant biomechanical stability at early implantation times. *J Oral Maxillofac Surg*. 2013;71(2):69.
18. Cohen O, Ormianer Z, Tal H, Rothamel D, Weinreb M, Moses O. Differences in crestal bone-to-implant contact following an under-drilling compared to an over-drilling protocol. A study in the rabbit tibia. *Clin Oral Investig*. 2016;20(9):2475-2480.
19. Trisi P, Todisco M, Consolo U, Travaglini D. High versus low implant insertion torque: a histologic, histomorphometric, and biomechanical study in the sheep mandible. *Int J Oral Maxillofac Implants*. 2011;26(4):837-849.
20. Coelho PG, Suzuki M, Guimaraes MVM, et al. Early bone healing around different implant bulk designs and surgical techniques: A study in dogs. *Clin Implant Dent Relat Res*. 2010;12(3):202-208.
21. Marin C, Granato R, Suzuki M, Gil JN, Janal MN, Coelho PG. Histomorphologic and histomorphometric evaluation of various endosseous implant healing chamber configurations at early implantation times: A study in dogs. *Clin Oral Implants Res*. 2010;21(6):577-583.
22. Yurttutan ME, Kestane R, Keskin A, Dereci O. Biomechanical evaluation of oversized drilling on implant stability - an experimental study in sheep. *J Pak Med Assoc*. 2016;66(2):147-150.
23. Freitas AC, Bonfante EA, Giro G, Janal MN, Coelho PG. The effect of implant design on insertion torque and immediate micromotion. *Clin Oral Implants Res*. 2012;23(1):113-118.
24. Akça K, Kökat AM, Cömert A, Akkocaoğlu M, Tekdemir I, Cehreli MC. Torque-fitting and resonance frequency analyses of implants in conventional sockets versus controlled bone defects in vitro. *Int J Oral Maxillofac Surg*. 2010;39(2):169-173.
25. Rabel A, Kohler SG, Schmidt-Westhausen AM. Clinical study on the primary stability of two dental implant systems with resonance frequency analysis. *Clin Oral Investig*. 2007;11(3):257-265.

26. Khayat PG, Arnal HM, Tourbah BI, Sennerby L. Clinical outcome of dental implants placed with high insertion torques (up to 176 Ncm). *Clin Implant Dent Relat Res*. 2013;15(2):227-233.
27. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS med*. 2009;6(7):e1000097.
28. Schardt C, Adams MB, Owens T, Keitz S, Fontelo P. Utilization of the PICO framework to improve searching PubMed for clinical questions. *BMC Med Inform Decis Mak*. 2007;7:16.
29. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med (Zagreb)*. 2012;22(3):276-282.
30. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol*. 2014;14:43.
31. Berglundh T, Stavropoulos A. Preclinical in vivo research in implant dentistry. Consensus of the eighth European workshop on periodontology. *J Clin Periodontol*. 2012;39 Suppl 12:1-5.
32. Schwarz F, Iglhaut G, Becker J. Quality assessment of reporting of animal studies on pathogenesis and treatment of peri-implant mucositis and peri-implantitis. A systematic review using the ARRIVE guidelines. *J Clin Periodontol*. 2012;39 Suppl 12:63-72.
33. Vesterinen HM, Sena ES, Egan KJ, et al. Meta-analysis of data from animal studies: a practical guide. *J Neurosci Methods*. 2014;221:92-102.
34. Deeks JJ, Higgins JP, Altman DG. Analysing Data and Undertaking Meta-Analyses. In: Higgins JP, Green S, eds. *Cochrane Handbook for Systematic Reviews of Interventions*. John Wiley & Sons, Ltd; 2008:243-296.
35. Jung U, Kim S, Kim Y, Cha J, Lee I, Choi S. Osseointegration of dental implants installed without mechanical engagement: a histometric analysis in dogs. *Clin Oral Implants Res*. 2012;23(11):1297-1301.
36. Rea M, Lang NP, Ricci S, Mintrone F, González González G, Botticelli D. Healing of implants installed in over- or under-prepared sites--an experimental study in dogs. *Clin Oral Implants Res*. 2015;26(4):442-446.
37. Jimbo R, Tovar N, Yoo DY, Janal MN, Anchieta RB, Coelho PG. The effect of different surgical drilling procedures on full laser-etched microgrooves surface-treated implants: an experimental study in sheep. *Clin Oral Implants Res*. 2014;25(9):1072-1077.
38. Kim M, Jung U, Kim S, Lee J, Lee I, Choi S. Bone apposition on implants coated with calcium phosphate by ion beam assisted deposition in oversized drilled sockets: a histologic

- and histometric analysis in dogs. *Journal of Periodontal and Implant Science*. 2013;43(1):18-23.
39. Meredith N. Assessment of implant stability as a prognostic determinant. *Int J Prosthodont*. 1998;11(5):491-501.
 40. Trisi P, Perfetti G, Baldoni E, Berardi D, Colagiovanni M, Scogna G. Implant micromotion is related to peak insertion torque and bone density. *Clin Oral Implants Res*. 2009;20(5):467-471.
 41. Ottoni JMP, Oliveira ZFL, Mansini R, Cabral AM. Correlation between placement torque and survival of single-tooth implants. *International Journal of Oral and Maxillofacial Implants*. 2005;20(5):769-776.
 42. Testori T, Del Fabbro M, Capelli M, Zuffetti F, Francetti L, Weinstein RL. Immediate occlusal loading and tilted implants for the rehabilitation of the atrophic edentulous maxilla: 1-year interim results of a multicenter prospective study. *Clin Oral Implants Res*. 2008;19(3):227-232.
 43. Meredith N, Shagaldi F, Alleyne D, Sennerby L, Cawley P. The application of resonance frequency measurements to study the stability of titanium implants during healing in the rabbit tibia. *Clin Oral Implants Res*. 1997;8(3):234-243.
 44. Szmukler-Moncler S, Salama H, Reingewirtz Y, Dubruille JH. Timing of loading and effect of micromotion on bone-dental implant interface: review of experimental literature. *J Biomed Mater Res*. 1998;43(2):192-203.
 45. Lioubavina-Hack N, Lang NP, Karring T. Significance of primary stability for osseointegration of dental implants. *Clin Oral Implants Res*. 2006;17(3):244-250.
 46. Marin C, Bonfante EA, Jeong R, et al. Histologic and biomechanical evaluation of 2 resorbable-blasting media implant surfaces at early implantation times. *J Oral Implantol*. 2013;39(4):445-453.
 47. Shanbhag S, Shanbhag V, Stavropoulos A. Genomic analyses of early peri-implant bone healing in humans: a systematic review. *Int J Implant Dent*. 2015;1(1):5.
 48. Buser D, Broggini N, Wieland M, et al. Enhanced bone apposition to a chemically modified SLA titanium surface. *J Dent Res*. 2004;83(7):529-533. doi: 10.1177/154405910408300704.
 49. Wennerberg A, Galli S, Albrektsson T. Current knowledge about the hydrophilic and nanostructured SLActive surface. *Clinical, cosmetic and investigational dentistry*. 2011;3:59-67.

50. Wennerberg A, Jimbo R, Stübinger S, Obrecht M, Dard M, Berner S. Nanostructures and hydrophilicity influence osseointegration: a biomechanical study in the rabbit tibia. *Clin Oral Implants Res.* 2014;25(9):1041-1050.
51. Zhao G, Schwartz Z, Wieland M, et al. High surface energy enhances cell response to titanium substrate microstructure. *J Biomed Mater Res A.* 2005;74(1):49-58.
52. Sul YT, Kang BS, Johansson C, Um HS, Park CJ, Albrektsson T. The roles of surface chemistry and topography in the strength and rate of osseointegration of titanium implants in bone. *J Biomed Mater Res A.* 2009;89(4):942-950.
53. Leonard G, Coelho P, Polyzois I, Stassen L, Claffey N. A study of the bone healing kinetics of plateau versus screw root design titanium dental implants. *Clin Oral Implants Res.* 2009;20(3):232-239.
54. Baldassarri M, Bonfante E, Suzuki M, et al. Mechanical properties of human bone surrounding plateau root form implants retrieved after 0.3-24 years of function. *J Biomed Mater Res B Appl Biomater.* 2012;100(7):2015-2021.
55. Gil LF, Suzuki M, Janal MN, et al. Progressive plateau root form dental implant osseointegration: A human retrieval study. *J Biomed Mater Res B Appl Biomater.* 2015;103(6):1328-1332.
56. Piattelli A, Artese L, Penitente E, et al. Osteocyte density in the peri-implant bone of implants retrieved after different time periods (4 weeks to 27 years). *J Biomed Mater Res B Appl Biomater.* 2014;102(2):239-243.
57. Coelho PG, Bonfante EA, Marin C, Granato R, Giro G, Suzuki M. A human retrieval study of plasma-sprayed hydroxyapatite-coated plateau root form implants after 2 months to 13 years in function. *J Long Term Eff Med Implants.* 2010;20(4):335-342.
58. Chowdhary R, Halldin A, Jimbo R, Wennerberg A. Evaluation of stress pattern generated through various thread designs of dental implants loaded in a condition of immediately after placement and on osseointegration--an FEA study. *Implant Dent.* 2013;22(1):91-96.
59. Alves C, Neves M. Tapered implants: from indications to advantages. *Int J Periodontics Restorative Dent.* 2009;29(2):161-167.
60. Lima de Andrade C, Carvalho MA, Bordin D, da Silva WJ, Del BC, Sotto-Maior B. Biomechanical Behavior of the Dental Implant Macrodesign. *Int J Oral Maxillofac Implants.* 2017;32(2):264-270.
61. Coelho PG, Granato R, Marin C, et al. The effect of different implant macrogeometries and surface treatment in early biomechanical fixation: an experimental study in dogs. *J Mech Behav Biomed Mater.* 2011;4(8):1974-1981.

62. Coelho PG, Jimbo R, Tovar N, Bonfante EA. Osseointegration: hierarchical designing encompassing the micrometer, micrometer, and nanometer length scales. *Dent Mater.* 2015;31(1):37-52.
63. Chang PK, Chen YC, Huang CC, Lu WH, Chen YC, Tsai HH. Distribution of micromotion in implants and alveolar bone with different thread profiles in immediate loading: a finite element study. *Int J Oral Maxillofac Implants.* 2012;27(6):96.
64. Stavropoulos A, Cochran D, Obrecht M, Pippenger BE, Dard M. Effect of Osteotomy Preparation on Osseointegration of Immediately Loaded, Tapered Dental Implants. *Adv Dent Res.* 2016;28(1):34-41.
65. Pearce AI, Richards RG, Milz S, Schneider E, Pearce SG. Animal models for implant biomaterial research in bone: a review. *Eur Cell Mater.* 2007;13(1):1-10.
66. Sartoretto SC, Uzeda MJ, Miguel FB, Nascimento JR, Ascoli F, Calasans-Maia MD. Sheep as an experimental model for biomaterial implant evaluation. *Acta ortopedica brasileira.* 2016;24(5):262-266.
67. Stadlinger B, Pourmand P, Locher MC, Schulz MC. Systematic review of animal models for the study of implant integration, assessing the influence of material, surface and design. *J Clin Periodontol.* 2012;39 Suppl 12:28-36.
68. Aerssens J, Boonen S, Lowet G, Dequeker J. Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. *Endocrinology.* 1998;139(2):663-670.
69. Lekholm U, Zarb GA. Patient selection and preparation. In: Brånemark PI, Zarb GA, Albrektsson T, eds. *Tissue-integrated prosthesis: osseointegration in clinical dentistry.* Chicago: Quintessence Publ Co; 1985:199-209.
70. Stavropoulos A, Sculean A, Bosshardt DD, Buser D, Klinge B. Pre-clinical in vivo models for the screening of bone biomaterials for oral/craniofacial indications: focus on small-animal models. *Periodontol 2000.* 2015;68(1):55-65.
71. Vignoletti F, Abrahamsson I. Quality of reporting of experimental research in implant dentistry. Critical aspects in design, outcome assessment and model validation. *J Clin Periodontol.* 2012;39 Suppl 12:6-27.

Tables

TABLE 1 General Information of the included preclinical studies

Study	Objective(s)	Evaluation and sample size	Animal model (gender, strain, age, n/group and location)	Implant system	Implant material and surface treatment	Implant geometry and dimensions (L x Ø: mm)
Campos <i>et al.</i> (2012)	To evaluate the effect of drilling dimensions on IT and early implant osseointegration stage	Biologic + Biomechanical	6 adult male Beagle dogs (n=6) 17 months (n=6) Extra-oral (radial)	Colosso (Emflis®, Itu, Brazil)	CP Ti (Grade II) GBA surface	Conical (10 x 3.4) Trapezoidal threads
Jung <i>et al.</i> (2012)	To elucidate the healing pattern of SLA-surface implant at two healing periods in a model that presents loosened implants installed without mechanical engagement	Biologic	5 male Mongrel dogs (n=5) 18-24 months (n=5) Intra-oral (mandible)	Implantium (Dentium®, Seoul, Korea)	CP Ti (Grade IV) SLA surface	Cylindrical (10 x 3.4) Progressive threads
Coelho <i>et al.</i> (2013)	To investigate the relationship between IT and RT of implants placed in bone sites drilled to different dimensions. To evaluate the effect of drilling dimensions for implant placement at early observation periods	Biologic + Biomechanical	6 adult male Beagle dogs (n=6) 17 months (n=6) Extra-oral (radial)	Colosso (Emflis®, Itu, Brazil)	CP Ti (Grade II) GBA surface	Conical (10 x 4.0) Trapezoidal threads
Kim <i>et al.</i> (2013)	To evaluate the osseointegration of CP-coated implants by ion beam assisted deposition with a lack of primary stability in different drilling protocols	Biologic	5 male Mongrel dogs (n=5) 18-24 months (n=5) Intra-oral (mandible)	Implantium (Dentium®, Seoul, Korea)	CP Ti (Grade IV) SLA-CaPO surface	Cylindrical (10 x 3.4) Progressive threads
Jimbo <i>et al.</i> (2014)	To evaluate the influence of drilling protocol on the early osseointegration of fully laser etched microgrooves implant surfaces	Biologic + Biomechanical	6 Finish Dorset crossbred sheep (n=12) 17 months (n=12) Extra-oral (iliac crest)	Laser-Lok® (BioHorizons®, Birmingham, AL, USA)	Ti-6AL-4V ELI Alloy (Grade V) LM surface	Tapered (8.0 x 4.5) Triangular threads
Baires-Campos <i>et al.</i> (2015)	To evaluate two different implant systems, a classic thread design versus another specific system designed for healing chamber formation along with primary stability, placed into two drilling schemes	Biologic + Biomechanical	10 Beagle dogs (n=10) 17 months (n=10) Extra-oral (tibia)	Unilite and SW (SIN®, São Paulo, SP, Brazil)	CP Ti (Grade IV) Dual AE surface	Conical (10 x 4.1) Trapezoidal threads Unitite: dual-thread SW: single thread design
Campos <i>et al.</i> (2015)	To determine the effect of three drilling protocols on biologic responses to a single implant design at early healing periods	Biologic + Biomechanical	10 Beagle dogs (n=10) 17 months (n=10) Extra-oral (tibia)	Unilite (SIN®, São Paulo, SP, Brazil)	CP Ti (Grade IV) Dual AE surface	Conical (10 x 4.1) Trapezoidal threads Unitite: dual-thread
Duyck <i>et al.</i> (2015)	To evaluate the role of implant insertion torque on the peri-implant bone healing during implant osseointegration	Biologic + Biomechanical	5 NZW rabbits (n=10) 4 months (n=10) Extra-oral (tibia)	JNE (GC Tech Europe GmbH, Brackerfeld, Germany)	CP Ti (Grade II) GBA surface	Conical (8.0 x 3.9) Trapezoidal threads

Rea <i>et al.</i> (2015)	To study bone healing at implants installed with different IT ranging from close to 0 Ncm to ≥ 70 Ncm	Biologic	6 Labrador dogs 48 months (n=6) Intra-oral (mandible)	GSII® (Osstem, Seoul, South Korea)	CP Ti (Grade IV) RBM surface	Conical (10 x 4.0) Triangular threads
Cohen <i>et al.</i> (2016)	To compare BIC between implants inserted at high torque (>35 Ncm) due to under-drilling of the crestal bone to those inserted at low torque (<10 Ncm) due to over-drilling of the crestal bone	Biologic	10 male NZW rabbits 6 months (n=10) Extra-oral (tibia)	SPi® and ICE® (Alpha Biotech, Petah, Tikva, Israel)	Ti-6AL-4V Alloy (Grade V) SAE surface	Conical (SPi®: 8.0 x 3.75; ICE®: 8.0 x 3.55) Progressive threads
Eom <i>et al.</i> (2016)	To investigate the influence of implant and drill diameters on the stability of implant and bone response	Biologic + Biomechanical	10 male miniature pigs 24 months (n=6) Intra-oral (mandible)	GSII® (Osstem, Seoul, South Korea)	CP Ti (Grade IV) RBM surface	Conical (8.5 x 3.5) Triangular threads
Yurtutan <i>et al.</i> (2016)	To evaluate the effect of oversized drilling on implant success and secondary stability	Biomechanical	2 female sheep 14 months (n=16) Extra-oral (tibia)	Genamer®, Ankara, Turkey	CP Ti (Grade IV) S surface	Cylindrical (10 x 4.0)

AL: aluminium; CP Ti: commercially pure titanium; dual AE: dual acid-etching; GBA: grit-blasting and acid-etching; LM: laser-treated microgroove in the coronal portion and machined surface along the implant body; NZW: New Zealand White rabbit; RBM: resorbable blasting media; S: sandblasting alone; SAE: sandblasting and acid-etching; SLA: sandblasting, large-grit, acid-etching; SLA-CaPO: SLA surface coated with calcium phosphate; V: vanadium.

TABLE 2 Checklist for quality assessment of *in vivo* studies according to modified ARRIVE guidelines and a predefined grading system

ARRIVE item	Introduction							Methods							Results				Discussion			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
Campos <i>et al.</i> (2012)	1	2	2	1	1	1	2	1	0	1	0	2	2	1	2	2	2	1	2	0		
Jung <i>et al.</i> (2012)	1	1	2	1	1	1	2	1	0	1	0	1	2	1	2	2	2	1	2	2		
Coelho <i>et al.</i> (2013)	1	2	2	1	1	1	2	1	0	2	0	2	2	1	2	1	2	1	2	2		
Kim <i>et al.</i> (2013)	1	1	2	1	1	1	1	1	0	1	0	1	1	1	2	2	2	1	2	2		
Jimbo <i>et al.</i> (2014)	1	2	2	1	1	1	2	1	2	2	0	2	2	1	2	2	2	2	2	2		
Baires-Campos <i>et al.</i> (2015)	1	2	2	1	1	1	2	1	0	1	0	2	2	1	2	2	2	1	2	1		
Campos <i>et al.</i> (2015)	1	2	2	1	2	1	2	1	0	1	0	2	2	1	2	2	2	1	2	0		
Duyck <i>et al.</i> (2015)	1	2	2	1	1	1	2	1	0	2	1	2	2	1	2	1	2	1	2	2		
Rea <i>et al.</i> (2015)	1	2	1	0	1	1	2	1	0	1	0	2	2	1	2	2	2	1	2	2		
Cohen <i>et al.</i> (2016)	1	2	1	1	1	1	1	1	0	1	0	2	2	1	1	1	0	1	2	2		
Eom <i>et al.</i> (2016)	1	1	2	0	0	1	2	1	0	2	0	2	2	1	2	1	2	1	2	2		
Yurttutan <i>et al.</i> (2016)	1	2	1	1	1	1	1	1	0	1	0	2	2	1	2	1	2	1	1	2		

A detailed list of ARRIVE items 1-20, together with scoring criteria, is provided in Table S6.

TABLE 3 Detailed information on the biomechanical outcomes of the included preclinical *in vivo* studies applied in the meta-analysis

Study	Surgical protocols				Time of healing	Biomechanical Parameters		Authors' Conclusions
	Implant Ø (mm)	Final Drill (mm)	IDD (mm)	IDD (%)		IT (N.cm)	RT (N.cm)	
Jimbo <i>et al.</i> (2014)	4.5	4.1 4.6	SD: 0.4 GAP: -0.1	8 -2	C: 6 w	Ø	98.67 ± 29.75 92.33 ± 27.83	Press-fit situation seemed to achieve higher secondary stability
Baires-Campos <i>et al.</i> (2015)	4.1	3.75 4.0	SD: 0.35 OD: 0.1	9 2	A: 2 w	43.00 ± 4.22 * 16.00 ± 3.91	Ø	Higher insertion torque values were observed for the smaller diameter instrumentation
Campos <i>et al.</i> (2015)	4.1	3.75 4.0	SD: 0.35 OD: 0.1	9 2	A: 2 w	40.00 ± 6.20 * 17.00 ± 7.90	Ø	The insertion torque values and osseointegration are not proportional
Yurtutan <i>et al.</i> (2016)	4.0	3.5 4.2	SD: 0.5 GAP: -0.2	12 -5	C: 12 w C: 12 w	Ø	97.75 ± 22.23 96.25 ± 21.93	Osseointegration can be achieved in the absence of primary stability

A: short-term healing (1-2 w); B: intermediate-term healing (3-4 w); C: long-term healing (≥ 5 w); GAP: friction-free oversized drilling (IDD ≤ -0.1 mm); IDD: implant-final drill discrepancy; IT: insertion torque; OD: stress-free oversized drilling (IDD = 0.0 - 0.1 mm); RT: removal torque; SD: press-fit/standard implant drilling technique (IDD = 0.2 - 0.4); UN: undersized implant drilling technique (IDD ≥ 0.5 mm); s.d.: standard deviation. Data is expressed by mean $\pm$ s.d. or median (95% confidence interval). * $P < 0.05$.

TABLE 4 Detailed information on the biologic outcomes of the included preclinical *in vivo* studies applied in the meta-analysis

Study	Surgical protocols			Histomorphometry		Descriptive histology	Authors' conclusion
	Implant Ø (mm)	Final drill (mm)	Healing period (w)	IDD (mm)	IDD (%)		
Campos <i>et al.</i> (2012)	3.4	3.2	A: 2 w	SD: 0.2	6	Extensive necrosis of CB	The compressive strain release of the oversized drilling could minimize the amount of necrotic and remodeling
			B: 4 w			Bone remodeling	
		3.5	A: 2 w	GAP: -0.1	3	Extensive necrosis of CB	
			B: 4 w			Bone remodeling	
Jung <i>et al.</i> (2012)	3.4	2.85	B: 4 w	UN: 0.55	16	Several voids in the CB	SLA surface implant installed in submerged healing with no mechanical engagement could achieve successful osseointegration
			C: 8 w			Bone remodeling	
		3.4	B: 4 w	OD: 0.0	0	NFB	
			C: 8 w			Mature lamellar bone; primary osteons	
Jimbo <i>et al.</i> (2014)	4.5	4.1	B: 3 w	SD: 0.4	8	Increased cBIC	Implants presented biocompatibility and osteoconduction
			C: 6 w			Comparable bone formation	
		4.6	B: 3 w	GAP: -0.1	-2	Greater NFB	
			C: 6 w			Comparable bone formation	
Baires-Campos <i>et al.</i> (2015)	4.1	3.75	A: 2 w	SD: 0.35	9	Necrotic bone and remodeling sites	The oversized-drilled sites presented increased bone formation
		4.0	OD: 0.1		2	HC filled with osteogenic tissue	
	4.1	3.75	A: 2 w	SD: 0.35	9	Necrotic bone and remodeling sites	
		4.0	GAP: -0.1		2	HC filled with osteogenic tissue	
Rea <i>et al.</i> (2015)	4.0	3.7	C: 16 w	SD: 0.3	8	All implants were well osseointegrated into mineralized bone irrespective of the surgical protocol applied	Implants installed in oversized sites may properly osseointegrate
		4	OD: 0.0		0		
		4.2	GAP: -0.2		-5		
Eom <i>et al.</i> (2016)	3.5	3.3	B: 3 w	SD: 0.2	6	Collagen deformation and micro cracks in CB	BIC and BD were high when using undersized drilling, but the speed of new bone formation was slow compared to oversized drilling
			C: 5 w				
		3.5	B: 3 w	OD: 0.0	0	No collagen deformation or micro cracks	
			C: 5 w				

A: short-term healing (1-2 w); B: intermediate-term healing (3-4 w); C: long-term healing (≥ 5 w); BD: bone density; BIC: bone-to-implant contact; CB: cortical bone; GAP: friction-free oversized drilling (IDD ≤ -0.1 mm); HC: healing chambers; IDD: implant-final drill discrepancy; NFB: newly formed bone/woven bone; OD: stress-free oversized drilling (IDD = 0.0 - 0.1 mm); SD: pressurized standard implant drilling technique (IDD = 0.2 - 0.4); UN: undersized implant drilling technique (IDD ≥ 0.5 mm); s.d.: standard deviation. Data is expressed by mean $\pm$ s.d. or median (95% confidence interval). * $P < 0.05$. Values in *italic* were not included in the meta-analysis.

Figures

Figure 1. Schematic illustration depicting the implant-last drill discrepancy (IDD; mm) between a tapered dental implant (3.5 mm, Ø) with progressive threads. Red dotted lines: outer implant diameter. Transparent blue, last drill dimension over imposed on the implant. (a) Undersized surgical preparation of the implant site (UN) with a 2.9-mm last drill depicting the mismatch of the inner and outer implant diameters in relation to the implantation bed wall (IDD = 0.6 mm). (b) Standard surgical protocol (press-fit condition; SD) with a 3.2-mm last drill and tapping of the host site performed with the implant as such while the threads engage within the bone (IDD = 0.3 mm). Oversized surgical preparations of the implant site (test OD) depicting (c) a stress-free implant insertion where the dimension of the bone wall is nearly similar to the implant outer diameter (IDD = 0.0 mm) and (d) a friction-free condition where a gap is produced between the implant and parent bone wall (IDD = -0.1 mm). Observe that the interplay between implant macrogeometry and oversized drilling technique of the implant site form healing chambers (void spaces) of varied dimensions between the implant bulk and the implantation site wall. The image of the implant was a courtesy of Neodent® (Curitiba – PR, Brazil).

Figure 2. PRISMA flow diagram illustrating the double-blind three-stage screening process: search strategy, study screening and final selection (n = number of articles). Interrater reliability (kappa coefficient; κ) describes the level of agreement between reviewers.

Figure 3. Risk of Bias (RoB) assessment presented as percentages across all included in vivo studies (n = 12) by applying the SYRCLE's RoB tool. The RoB was assessed as 'high' (no), 'unclear' or 'low' (yes). A detailed list of items 1-10 and respective signaling questions is provided in Table S5.

	1. Random sequence generation	2. Animal characteristics at baseline	3. Allocation concealment	4. Random housing of the animals	5. Blinding of caregivers and/or investigators	6. Calibrating of surgeons	7. Random outcome assessment	8. Blinding of outcome assessor	9. Incomplete data adequately addressed	10. Selective outcome reporting
Campos <i>et al.</i> (2012)	?	+	?	?	?	?	?	?	+	+
Jung <i>et al.</i> (2012)	?	+	?	?	?	?	?	?	+	+
Coelho <i>et al.</i> (2013)	?	+	?	?	?	?	?	?	+	+
Kim <i>et al.</i> (2013)	?	+	?	?	?	?	?	?	+	+
Jimbo <i>et al.</i> (2014)	?	+	?	?	?	?	?	+	+	+
Baires-Campos <i>et al.</i> (2015)	?	+	?	?	?	?	?	?	+	+
Campos <i>et al.</i> (2015)	?	+	?	?	?	?	?	?	+	+
Rea <i>et al.</i> (2015)	?	+	?	?	?	?	?	?	+	+
Cohen <i>et al.</i> (2015)	?	+	?	?	?	?	?	?	+	+
Duyck <i>et al.</i> (2015)	+	+	?	?	?	?	?	?	-	+
Eom <i>et al.</i> (2016)	?	+	?	?	?	?	?	?	+	+
Yurtutan <i>et al.</i> (2016)	?	+	?	?	?	?	?	?	+	+

Figure 4. Risk of Bias (RoB) summary as per authors' judgement about each item for each included in vivo study (n = 12) by applying the SYRCLE's RoB tool.

ITV (N/cm) - test OD

(a) Short-term healing - extra-oral

RTV (N/cm) - test GAP

(b) Long-term healing - extra-oral

Figure 5. Forest plot for the comparison of implants installed in sites prepared with standard drilling technique (control; $\text{IDD} \geq 0.2$ mm) and stress-free oversized drilling technique (test OD; $\text{IDD} = 0.0 - 0.1$ mm) and friction-free oversized implant drilling technique (test GAP; $\text{IDD} \leq -0.1$ mm) for the biomechanical outcomes of (a) insertion torque (ITV; N/cm), measured as the maximum torque employed during implant placement, and (b) removal torque (RTV; N/cm), as the maximum torque obtained in the unscrewing process in animal models. The forest plot displays the relative weight of the individual studies, the estimates of treatment effect (ES) expressed as weighted mean differences (WMD), 95% confidence intervals (CI) and a predictive interval for the outcomes. The diamond indicates the pooled estimate and its 95% CI.

%BIC - test OD

(a) Intermediate-term healing - intra-oral

(b) Long-term healing - intra-oral

Figure 6. Forest plot for the comparison of implants installed in sites prepared with standard to undersized drilling technique (control; IDD ≥ 0.2 mm) and stress-free oversized drilling technique (test OD; IDD = 0.0 – 0.1 mm) for the biologic outcome of bone-to-implant contact (%BIC), expressed as the relative amount of bone growth onto the implant surface within the implant site or within a region of interest in animal models, at (a) the intermediate- and (b) long-term healing. The forest plot displays the relative weight of the individual studies, the estimates of treatment effect (ES) expressed as weighted mean differences (WMD), 95% confidence intervals (CI) and a predictive interval for the outcomes. The diamond indicates the pooled estimate and its 95% CI.

%BD - test OD

(a) Short-term healing - extra-oral

(b) Intermediate-term healing - intra-oral

(c) Long-term healing - intra-oral

%BD - test GAP

(d) Intermediate-term healing - extra-oral

Figure 7. Forest plot for the comparison of implants installed in sites prepared with standard to undersized drilling technique (control; $\text{IDD} \geq 0.2$ mm) and stress-free oversized drilling technique (test OD; $\text{IDD} = 0.0 - 0.1$ mm) or friction-free oversized implant drilling technique (test GAP; $\text{IDD} \leq -0.1$ mm) for the biologic outcome of peri-implant bone density (%BD), expressed as the relative amount of the total area of mineralized bone within the implant site or within a region of interest in animal models, at different time-points. The forest plot displays the relative weight of the individual studies, the estimates of treatment effect (ES) expressed as weighted mean differences (WMD), 95% confidence intervals (CI) and a predictive interval for the outcomes. The diamond indicates the pooled estimate and its 95% CI.

Supplementary information

Figure S1. Characteristics of the study design of all eligible articles (n = 12).

TABLE S1 Terms and strategy used to search PubMed

Search no.	Search parameters	Results
#1	"models, animal"[MeSH Terms] OR "humans"[MeSH Terms]	16,429,954
#2	"dental implants"[MeSH Terms]	17,906
#3	"dental implant"[Title/Abstract] OR "drilling technique"[Title/Abstract] OR "implant design"[Title/Abstract] OR "drilling protocol"[Title/Abstract] OR overpreparation[Title/Abstract] OR overdrilling[Title/Abstract] OR "macrogeometry"[Title/Abstract]	6,369
#4	#2 OR #3	2,783
#5	"torque"[MeSH Terms] OR "osseointegration"[MeSH Terms] OR "histology"[MeSH Terms] OR "wound healing"[MeSH Terms] OR "osteogenesis"[MeSH Terms]	479,106
#6	"insertion torque"[Title/Abstract] OR "histomorphometry"[Title/Abstract] OR "implant stability"[Title/Abstract] OR "torque"[Title/Abstract] OR "biomechanical"[Title/Abstract] OR "bone-to-implant contact"[Title/Abstract] OR "bone density"[Title/Abstract] OR "removal torque"[Title/Abstract]	72,913
#7	#5 OR #6	540,855
Final search	#1 AND #4 AND #7	823

TABLE S2 Terms and strategy used to search Web of Knowledge

Search No.	Search Parameters	Results
#1	TITLE-ABS-KEY (osseointegration* OR dental implant*)	22,919
#2	TS=(drilling technique* OR drilling protocol* OR overpreparation OR overdrilling OR oversized OR macrogeometry*)	12,612
#3	TS=(torque OR histology OR osteogenesis OR implant stability OR biomechanical OR bone-to-implant contact OR bone density)	324,972
Final search	#1 AND #2 AND #3	150

TABLE S3 Terms and strategy used to search Scopus

Search No.	Search Parameters	Results
#1	TS (osseointegration* OR dental implant*)	47,725
#2	TS (drilling technique* OR drilling protocol* OR overpreparation OR overdrilling OR oversized OR macrogeometry*)	718
#3	TS (torque OR histology OR osteogenesis OR implant stability OR biomechanical OR bone-to-implant contact OR bone density)	2,290
Final search	#1 AND #2 AND #3	14

TABLE S4 Terms and strategy used to search Cochrane Library

Search No.	Search Parameters	Results
#1	MeSH descriptor: [dental implants] explode all trees	1,198
#2	MeSH descriptor: [osseointegration] explode all trees	326
#3	MeSH descriptor: [models, animal] explode all trees	407
#4	MeSH descriptor: [humans] explode all trees	1,426
#5	drilling technique:ti, ab, kw	199
#6	drilling protocol:ti, ab, kw	29
#7	oversized:ti, ab, kw	48
#8	MeSH descriptor: [torque] explode all trees	627
#9	MeSH descriptor: [histology] explode all trees	4,299
#10	MeSH descriptor: [osteogenesis] explode all trees	291
#11	insertion torque:ti, ab, kw	78
#12	implant stability:ti, ab, kw	552
#13	biomechanical:ti, ab, kw	2,884
#14	bone-to-implant contact:ti, ab, kw	29
#15	removal torque:ti, ab, kw	26
#16	bone density:ti, ab, kw	7,745
Final search	(#1 or #2 or #3 or #4) and (#5 or #6 or #7) and (#8 or #9 or #10) and (#11 or #12 or #13 or #14 or #15 or #16)	3

TABLE S5 The SYRCLE’s Risk of Bias (RoB) main items assessed in relation to low, high or unclear risk of bias (ROB)

SYRCLE item
1) Was the allocation sequence adequately generated and applied?
Did the investigators describe a random component in the sequence generation process?
2) Were the groups similar at baseline or were they adjusted for confounders in the analysis?
Was the distribution of relevant baseline characteristics balanced for the intervention and control groups?
3) Was the allocation to the different groups adequately concealed during?
Could the investigator allocating the animals to intervention or control group not foresee assignment due to one of the following or equivalent methods?
4) Were the animals randomly housed during the experiment?
Did the authors randomly place the cages or animals within the animal room/facility?
5) Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?
Was blinding of caregivers and investigators ensured, and was it unlikely that their blinding could have been broken?
6) Were the surgeons trained and calibrated in advance for the experimental surgeries?
Did the investigators receive appropriate training and calibration ahead of the experimental surgical procedures, including maxillary sinus floor augmentation and/or placement of dental implants?
7) Were animals selected at random for outcome assessment?
Did the investigators randomly pick an animal during outcome assessment, or did they use a random component in the sequence generation for outcome assessment?
8) Was the outcome assessor blinded?
Was blinding of the outcome assessor ensured, and was it unlikely that blinding could have been broken?
9) Were incomplete outcome data adequately addressed? (*)
Were all animals included in the analysis?
10) Are reports of the study free of selective outcome reporting? (*)
Was the study protocol available and were all of the study’s pre-specified primary and secondary outcomes reported in the current manuscript?

TABLE S6 Checklist for quality assessment of studies according to modified ARRIVE guidelines and a predefined grading system

Item	Description	Grade
1	Title	0 = inaccurate/not concise 1 = accurate and concise
2	Abstract Summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study	0 = clearly inaccurate 1 = possibly accurate 2 = clearly accurate
3	Introduction Background – objectives, experimental approach and rationale, relevance to human biology	0 = clearly insufficient 1 = possibly sufficient 2 = clearly sufficient
4	Introduction Objectives – primary and secondary (if applicable)	0 = not clear 1 = clear
5	Methods Ethical statement – nature of the review permission, relevant licences, national and institutional guidelines for the care and use of animals	0 = clearly insufficient 1 = possibly sufficient 2 = clearly sufficient
6	Methods Study design (a) number of experimental and control groups	0 = no 1 = yes
7	Methods Experimental procedure – precise details (i.e., how, when, where, why)	0 = clearly insufficient 1 = possibly sufficient 2 = clearly sufficient
8	Methods Experimental animals – species, strain, sex, developmental stage, weight, source of animals	0 = clearly insufficient 1 = possibly sufficient 2 = clearly sufficient
9	Methods Housing and husbandry – conditions and welfare-related assessments and interventions (i.e., type of cage, bedding material, number of cage companions, light/dark cycle, temperature, access to food and water)	0 = clearly insufficient 1 = possibly sufficient 2 = clearly sufficient
10	Methods Sample size – total number of animals used in each experimental group, details of calculation	0 = clearly inadequate 1 = possibly adequate 2 = clearly adequate
11	Methods Allocation animals to experimental groups – randomization or matching, order in which animals were treated and assessed	0 = no 1 = yes
12	Methods Experimental outcomes – definition of primary and secondary outcomes	0 = no 1 = unclear/not complete 2 = yes
13	Methods Statistical methods – details and unit of analysis	0 = no 1 = unclear/not complete 2 = yes
14	Results Baseline data – characteristics and health status of animals	0 = no 1 = yes
15	Results Numbers analysed – absolute numbers in each group included in each analysis, explanation for exclusion	0 = clearly inadequate 1 = possibly adequate 2 = clearly adequate

17	Results Adverse events – details and modifications for reduction	0 = no 1 = unclear/not complete 2 = yes
18	Discussion Interpretation/scientific implications – study limitations including animal model, implications for the 3Rs (replacement, refinement or reduction)	0 = clearly inadequate 1 = possibly adequate 2 = clearly adequate
19	Discussion Generalisability/translation – relevance to human biology	0 = clearly inadequate 1 = possibly adequate 2 = clearly adequate
20	Discussion Funding – sources, role of the funders	0 = clearly inadequate 1 = possibly adequate 2 = clearly adequate

Item 11 included information on randomization of animals to treatment groups.

TABLE S7 List of preclinical *in vivo* and *ex vivo* studies included in the systematic review

Author	Year	Title
Campos <i>et al.</i>	2012	Effect of drilling dimension on implant placement torque and early osseointegration stages - An experimental study in dogs
Jung <i>et al.</i>	2012	Osseointegration of dental implants installed without mechanical engagement - A histometric analysis in dogs
Coelho <i>et al.</i>	2013	Biomechanical evaluation of undersized drilling on implant biomechanical stability at early implantation times
Kim <i>et al.</i>	2013	Bone apposition on implants coated with calcium phosphate by ion beam assisted deposition in oversized drilled sockets: a histologic and histometric analysis in dogs
Jimbo <i>et al.</i>	2014	The effect of different surgical drilling procedures on full laser-etched microgrooves surface-treated implants: an experimental study in sheep
Baires-Campos <i>et al.</i>	2015	Drilling dimension effects in early stages of osseointegration and implant stability in a canine model
Campos <i>et al.</i>	2015	Are insertion torque and early osseointegration proportional? A histologic evaluation
Duyck <i>et al.</i>	2015	Effect of insertion torque on titanium implant osseointegration: an animal experimental study
Rea <i>et al.</i>	2015	Healing of implants installed in over- or under-prepared sites - An experimental study in dogs
Cohen <i>et al.</i>	2016	Differences in crestal bone-to-implant contact following an under-drilling compared to an over-drilling protocol. A study in the rabbit tibia
Eom <i>et al.</i>	2016	Effects of Different Implant Osteotomy Preparation Sizes on Implant Stability and Bone Response in the Minipig Mandible
Yurttutan <i>et al.</i>	2016	Biomechanical evaluation of oversized drilling on implant stability-an experimental study in sheep

TABLE S8 List of preclinical *in vivo* and *ex vivo* studies excluded in the systematic review

Author	Year	Title	Exclusion criteria
Moon <i>et al.</i>	2010	The effect of implant shape and bone preparation on primary stability	<i>Ex vivo</i> study
Kold <i>et al.</i>	2005	Bone compaction enhances fixation of hydroxyapatite-coated implants in a canine gap model	Field of Orthopedics
Stavropoulos <i>et al.</i>	2016	Effect of osteotomy preparation on osseointegration of immediately loaded, tapered dental implants	Evaluation of profiling of the cortical bone
Fernandes <i>et al.</i>	2008	Primary stability of turned and acid-etched screw-type implants: A removal torque and histomorphometric study in rabbits	Use of short implants (≤ 6 mm) and/or implants presenting different macrogeometry
Coelho <i>et al.</i>	2010	Early bone healing around different implant bulk designs and surgical techniques: A study in dogs	
Stocchero <i>et al.</i>	2018	Influence of different drilling preparation on cortical bone: A biomechanical, histological, and micro-CT study on sheep	
Blanco <i>et al.</i>	2011	Influence on early osseointegration of dental implants installed with two different drilling protocols: A histomorphometric study in rabbit	Absence of an oversized drilling technique according to the present study protocol
Jung <i>et al.</i>	2014	Secondary stability of microthickness hydroxyapatite-coated dental implants installed without primary stability in dogs	

TABLE S9 Detailed information on the biomechanical outcomes of the included preclinical *In vivo* studies

Study	Surgical protocols			Biomechanical Parameters				Authors' Conclusions
	Implant Ø (mm)	Final Drill (mm)	IDD (mm)	IDD (%)	Time of healing	%ISQ	IT (N.cm)	RT (N.cm)
Campos <i>et al.</i> (2012)	3.4	3.2	SD: 0.2	6			160.00 ± 25.00 * $\varnothing$	
		3.5	GAP: -0.1	-3			130.00 ± 40.00	
		3.8	GAP: -0.4	-12			70.00 ± 8.00 $\varnothing$	
Coelho <i>et al.</i> (2013)	4.0	3.2	UN: 0.8	20	A: 1 w		92.00 ± 21.00	79.00 ± 30.00 * $\varnothing$, f, β , α
					B: 3 w		99.00 ± 35.00	70.00 ± 23.00 * $\varnothing$, f, β , α
		3.5	SD: 0.5	12	A: 1 w		85.00 ± 20.00	75.00 ± 20.00 $\varnothing$
					B: 3 w		70.00 ± 23.00	82.00 ± 15.00 f
		3.8	OD: 0.2	5	A: 1 w		58.00 ± 33.00	60.00 ± 38.00 β
					B: 3 w		65.00 ± 37.00	65.00 ± 36.00 α
Jimbo <i>et al.</i> (2014)	4.5	3.2	UN: 1.3	29	B: 3 w		107.83 ± 32.51 * $\varnothing$, f	Press-fit situation seemed to achieve higher secondary stability
					C: 6 w		106.83 ± 32.21	
		3.7	UN: 0.8	18	B: 3 w		56.83 ± 17.13 $\varnothing$	
					C: 6 w		92.67 ± 27.94	
		4.1	SD: 0.4	8	B: 3 w		$\varnothing$	91.67 ± 27.63 * $\varnothing$, f
					C: 6 w		98.67 ± 29.75	
		4.6	GAP: -0.1	-2	B: 3 w		71.17 ± 21.45 f	
Baires-Campos <i>et al.</i> (2015)	4.1	3.75	SD: 0.35	9	SW		45.00 ± 4.22 * $\varnothing$	Higher insertion torque values were observed for the smaller diameter instrumentation
					Unitle		43.00 ± 4.22 * f	
		4.0	OD: 0.1	2	SW		13.00 ± 2.76 $\varnothing$	
Campos <i>et al.</i> (2015)					Unitle		16.00 ± 3.91 f	
	4.1	3.5	UN: 0.6	15			73.00 ± 10.90 * $\varnothing$	The insertion torque values and osseointegration are not proportional
		3.75	SD: 0.35	9			40.00 ± 6.20 $\varnothing$, * f	
		4.0	OD: 0.1	2			17.00 ± 7.90 f	

Eom <i>et al.</i> (2016)	3.5	3.0	UN: 0.5	14	A: 0 w A: 1w C: 3 w D: 5 w	82.60 ± 4.50 * Δ 83.00 ± 0.00 84.20 ± 4.70 85.00 ± 0.00	> 50.00 * Δ , f	Primary and secondary implant stability were high when using a final drill that was smaller than the root diameter of the implant thread compared with a wide final drill
		3.3	SD: 0.2	6	A: 0 w A: 1w C: 3 w D: 5 w	78.30 ± 6.00 79.00 ± 0.00 84.50 ± 6.70 85.00 ± 0.00	32.70 ± 13.50 Δ	
		3.5	OD: 0.0	0	A: 0 w A: 1w C: 3 w D: 5 w	60.60 ± 14.70 Δ 66.00 ± 54.00 81.10 ± 8.40 79.00 ± 20.00	3.30 ± 3.00 f	
Yurtutan <i>et al.</i> (2016)	4.0	3.5	SD: 0.5	12	A: 0 w C: 12 w	56.87 ± 3.24 * Δ 60.50 ± 7.61		Osseointegration can be achieved in the absence of primary stability
		4.2	GAP: -0.2	-5	A: 0 w C: 12 w	0.00 ± 0.003 60.25 ± 7.46	97.75 ± 22.23 96.25 ± 21.93	

A: short-term healing (1-2 w); B: intermediate-term healing (3-4 w); BT IV: bone type II; BT IV: bone type IV; C: long-term healing (≥ 5 w); GAP: friction-free oversized drilling (IDD ≤ -0.1 mm); IDD: implant-final drill discrepancy; IT: insertion torque; ISQ: implant stability quotient; OD: stress-free oversized drilling (IDD = 0.0 - 0.1 mm); RT: removal torque; SD: press-fit/standard implant drilling technique (IDD = 0.2 - 0.4); UN: undersized implant drilling technique (IDD ≥ 0.5 mm); s.d.: standard deviation. Data is expressed by mean $\pm$ s.d. or median (95% confidence interval), * $P < 0.05$.

TABLE S10 Detailed information on the biologic outcomes of the included preclinical *in vivo* studies

Study	Surgical protocols			Histomorphometry			Descriptive histology	Authors' conclusion
	Implant Ø (mm)	Final Drill (mm)	IDD (mm)	IDD (%)	Healing period	%BIC	%BD	
Campos <i>et al.</i> (2012)	3.4	3.2	SD: 0.2	6	A: 2 w B: 4 w	38.00 ± 10.00 δ 65.00 ± 8.00 * δ	42.00 ± 20.00 62.00 ± 12.00	A greater amount of necrotic tissue decreases implant stability over time until secondary stability. The compressive strain release of the OD could minimize the amount of necrotic bone and remodelling SLA surface implant installed in submerged healing with no mechanical engagement could achieve successful osseointegration A different pattern of healing and interfacial remodelling was observed between the different groups
		3.5	GAP: -0.1	-3	A: 2 w B: 4 w	40.00 ± 12.00 <i>f</i> 62.00 ± 5.00 * <i>f</i>	45.00 ± 20.00 55.00 ± 3.00	
		3.8	GAP: -0.4	-12	A: 2 w B: 4 w	40.00 ± 10.00 β 61.00 ± 7.00 * β	40.00 ± 20.00 58.00 ± 4.00	
							HC filled with osteogenic tissue HC with extensive NFB	
Jung <i>et al.</i> (2012)	3.4	2.85	UN: 0.55	16	B: 4 w C: 8 w	76.13 ± 7.98 74.34 ± 29.81	62.29 ± 14.76 54.90 ± 23.09	Several voids in the CB Bone remodeling NFB Mature lamellar bone; primary osteons
		3.4	OD: 0.0	0	B: 4 w C: 8 w	67.94 ± 18.49 75.51 ± 18.07	64.87 ± 14.90 46.94 ± 10.09	
	4.0	3.2	UN: 0.8	20	A: 1 w B: 3 w			
		3.5	SD: 0.5	12	A: 1 w B: 3 w			
Coelho <i>et al.</i> (2013)		3.8	OD: 0.2	5	A: 1 w B: 3 w	$\emptyset$	$\emptyset$	Extensive necrotic bone Bone remodeling Extensive necrotic bone Bone remodeling HC filled with osteogenic tissue Extensive NFB
Kim <i>et al.</i> (2013)	3.4	2.85	SD: 0.55	16	B: 4 w C: 8 w	78.09 69.52	64.62 57.98	Successful osseointegration could be achieved under unfavorable conditions without primary stability
		3.4	OD: 0.0	0	C: 4 w D: 8 w	64.43 77.38	42.63 58.54	
Jimbo <i>et al.</i> (2014)	4.5	3.2	UN: 1.3	29	B: 3 w C: 6 w	51.03 ± 15.38 * δ 64.56 ± 19.46 * <i>f</i> , β	45.19 ± 13.62 56.25 ± 16.96 * δ , <i>f</i>	Laser-etched microgrooved implant surface presented biocompatibility and osteoconduction
		3.7	UN: 0.8	18	B: 3 w C: 6 w	50.53 ± 15.23 * δ 60.99 ± 18.38 * <i>f</i> , β	49.00 ± 14.77 58.09 ± 17.51 * δ , <i>f</i>	

Eom <i>et al.</i> (2016)	3.5	3	UN: 0.5	14	A: 0 w A: 1 w C: 3 w D: 5 w	85.50 ± 2.60 * Δ , f 85.50 ± 2.60 * β , $\dagger$ 87.00 ± 13.00 * $\circ\phi$ 80.00 ± 11.60	85.40 ± 0.70 * Δ , f 90.00 ± 0.00 * β , $\dagger$ 88.00 ± 0.00 * $\circ\phi$ 80.60 ± 15.00	Collagen deformation and micro cracks in the CB	BIC and BD were high when applying undersized drilling, but the speed of new bone formation was relatively slow compared to oversized drilling
	3.3	6	SD: 0.2	6	A: 0 w A: 1 w C: 3 w D: 5 w	56.00 ± 7.40 Δ , * f 46.00 ± 27.00 β , * $\dagger$ 71.00 ± 44.00 * $\circ\phi$ 73.20 ± 18.10	47.70 ± 20.90 Δ , * f 58.00 ± 22.00 β , * $\dagger$ 68.00 ± 51.00 * $\circ\phi$ 66.40 ± 13.20	No collagen deformation or micro cracks in oversized implant sites	
	3.5	0	OD: 0.0	0	A: 0 w A: 1 w C: 3 w D: 5 w	0.00 ± 0.00 f 0.00 ± 0.00 $\dagger$ 25.00 ± 32.00 $\circ\phi$ 66.60 ± 14.90 * $\circ\phi$	0.00 ± 0.00 f 0.00 ± 0.00 $\dagger$ 15.00 ± 25.00 $\circ\phi$ 51.70 ± 17.50 * $\circ\phi$		

A: short-term healing (1-2 w); B: intermediate-term healing (3-4 w); C: long-term healing (≥ 5 w); BD: bone density; BIC: bone-to-implant contact; cBIC: coronal bone-to-implant contact; tBIC: total bone-to-implant contact; CB: cortical bone; GAP: friction-free oversized drilling (IDD ≤ -0.1 mm); HC: healing chambers; IDD: implant-final drill discrepancy; NFB: newly formed bone/woven bone; OD: stress-free oversized drilling (IDD = 0.0 – 0.1 mm); SD: press-fit/standard implant drilling technique (IDD = 0.2 – 0.4); UN: undersized implant drilling technique (IDD ≥ 0.5 mm), s.d.: standard deviation. Data is expressed by mean $\pm$ s.d. or median (95% confidence interval); * $P < 0.05$.

V

Effect of oversized implant site preparation on osseointegration of submerged tapered titanium implants: a study in the minipig mandible

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Abstract

Objective: To evaluate the effect of oversized surgical preparation of the implant site on marginal peri-implant bone level (MBL) and histomorphometrical parameters of osseointegration at tapered implants with a progressive thread geometry, compared to implants installed with a standard drilling protocol in a large intra-oral animal platform.

Materials and Methods: Ten hydrophilic implants (11.5 mm x 3.5 mm, L x Ø) were inserted in the edentulous mandible of female minipigs, by applying different drilling protocols (standard [SD, control] vs. oversized [OD, test]). SD included a 3-step series of drills without profiling of the cortical bone (IDD = 0.3 mm). OD included a 5-step series of drills and profiling of the cortical bone which resulted, respectively, in two IDD along the implant axis: (1) IDD = 0.1 mm in the 8-mm central apical length of the osteotomy; and (2) IDD = 0.0 mm in the 3.5-mm coronal length. Maximum insertion torque values (ITV; N.cm) were recorded and non-submerged healing was allowed for 12 weeks. Following termination, mandibular blocks were harvested for non-decalcified histomorphometric evaluation.

Results: OD produced statistically significant lower ITV and it showed reduced marginal bone changes, however without significant differences when compared to SD. In contrast, OD showed statistically significant higher values of bone-to-implant (BIC; %) and peri-implant bone density (BD; %) compared to SD ($P < 0.05$).

Conclusion: Oversized surgical preparation of the implant site yielded low insertion torque of a tapered implant presenting a hydrophilic surface technology, but enhanced osseointegration and peri-implant bone density, and preserved better the MBL.

Keywords: dental implant, surgical protocol, oversized drilling, insertion torque, bone-to-implant contact, minipigs

Introduction

Primary implant stability (*i.e.*, the initial rotational resistance of the implant as measured by maximum insertion torque values [ITV; N.cm]) is considered a prerequisite for successful osseointegration (*i.e.*, achievement of secondary/biological implant stability)^{1,2}. Among multiple variables, including bone density³, the interplay between implant macrogeometry (*e.g.*, body shape and thread design) and surgical technique (*e.g.*, final osteotomy drilling) has been shown to have a significant impact on the interfacial biomechanical strength^{4,5}. During early peri-implant healing, a gradual decline in the level of implant stability is expected to befall due to primary interfacial bone remodelling in sites initially in direct contact to the surface^{6,7}.

In order to attain high primary stability, mainly in sites with low-density bone, lateral compression of the surrounding tissue is performed by means of smaller-sized drills as per standard (press-fit condition with the dimension of the site matching the inner implant diameter while the threads engage within the bone) or undersized drilling protocols (mismatch of the inner and outer implant diameters in relation to the implantation bed walls)⁸. As far as thick poorly vascularized cortical and/or highly dense trabecular bone are concerned, excessive frictional and compressive forces beyond the physiological bone strain produce micro-fractures and impairment of microcirculation, disturbing the overall cellular activity and bone metabolism^{9,10}. This phenomenon triggers excessive bone remodelling of necrotic bone associated with low appositional rate (2 - 5 $\mu\text{m/day}$), which results in substantial decrease of implant stability mainly between 2 and 4 weeks *in vivo*^{11,12} and eventual crestal peri-implant bone loss (CBL)¹³⁻¹⁵.

On the other hand, larger final osteotomy drilling in interplay with implant macrogeometry aims to achieve either similar dimension between the bone defect and the implant outer diameter or over dimensioned sites, so-called oversized drilling technique^{5,16}. This protocol creates closed healing chambers (*i.e.*, void spaces) in the inner thread region by employing tapping and profiling (*i.e.*, enlargement of the marginal aspect) in the osteotomy^{16,17}. Healing chambers were shown to allow blood clot stabilization between the implant inner diameter and the bone wall, thereby creating an osteoprotective microenvironment that supports intramembranous-type healing mode with a bone appositional rate of 30 - 50 $\mu\text{m/day}$ ¹¹, hastening secondary stability already in the first week up to 6 weeks *in vivo*^{9,12}.

Despite current evidence supporting osseointegration of implants installed in over-drilled sites in various preclinical animal models^{5,9,12,16,18-22}, studies reporting on marginal peri-implant bone levels (MBL) of highly dense bone are limited and warrant further investigation. Thus, the objective of this preclinical *in vivo* study was to histomorphometrically evaluate the effect of an oversized implant site on MBL and parameters of osseointegration of tapered titanium implants with a progressive thread geometry, compared to implants installed with a standard drilling protocol in a large intra-oral animal platform. Specifically, it was hypothesized that an oversized drilling technique would promote higher amount and better quality of osseointegration in comparison to a press-fit condition.

Materials and Methods

The present preclinical *in vivo* study is reported according to the ARRIVE guidelines regarding all relevant items²³. The animal experimental protocol was approved by the General Directorate of Rural Economy and Veterinary Medicine, Department of Animal Health and Veterinary Welfare, Drugs and Applications (protocol no. 449328-2583), Region of Central Macedonia, Thessaloniki, Greece. Throughout the trials, handling and intervention of the animals followed the Federation for Laboratory Animal Science Associations (FELASA) guidelines (Directive 2010/63/EU). The experimental period of the study was conducted between January and April 2018.

Experimental units

Ten commercially pure titanium (CpTi) grade IV implants (Neodent®, Curitiba - PR, Brazil) Helix GM® (HLX; 11.5 mm x 3.5 mm, L x Ø) were used in the present study. HLX is a full dual tapered implant with a hybrid contour, cylindrical on the coronal part and conical on the apical area, where it contains an active apex and helicoidal flutes (Figure 1a). All implants present surface hydrophilicity (marketed as Acqua™; SAE-HD) with moderately rough microtopography (Figure 1b)²⁴. Additional details on the trial, including proprietary technology treatment, housing and husbandry, anaesthesia protocol, teeth extraction and histotechnical procedure, are found as supplementary information.

Experimental design

One implant system was used in the present study: Helix GM® Acqua™. Implant bed was prepared by applying two different surgical drilling protocols:

- (1) Standard drilling (SD; control group, n = 5): press-fit condition with the dimension of the site nearly matching the inner implant diameter while the threads engage within the bone.
- (2) Oversized drilling (OD; test group, n = 5): over dimensioned site instrumented with a larger last drill nearly matching the dimension between the bone defect and the implant outer diameter.

Drilling dimensions are further described in the subsection “drilling protocols”, according to diagram in Figure 2.

Experimental animals

This study included five female minipigs from a mixed breed, with an average of 2 years of age and weighting between 50 to 60 kg. All animals were bred in the laboratory animal facility at the Department of Veterinary Medicine in the Aristotle University of Thessaloniki for the single purpose to be used for experimental research under a rigorous health monitoring program. Aiming to guarantee selection blindness during the experimental allocation, control and experimental groups were randomly distributed in the mandible arch of each minipig in a split-mouth design, according to a computer-generated randomization list.

Surgical procedure

After acclimatization in the facilities for 2 weeks, surgical procedures were performed under general anaesthesia and aseptic conditions by one experienced surgeon (A.S.) in an animal operating room as previously reported elsewhere¹⁴.

Briefly, a mid-crestal incision in both sides of the edentulous posterior mandible was made to raise full thickness buccal and lingual flaps. Aiming a more accurate implant placement, the residual alveolar ridge was flattened by means of rotating instruments under copious sterile saline irrigation. Four implants were inserted in each side of the mandible (n = 8 implants/animal) with a drilling speed of 1.200 rpm under copious sterile saline irrigation. A total of 10 non-submerged implants were analysed in the present study – the remaining 30 devices are related to a different macrogeometry and loading condition, not allowing comparisons herein. Implants were positioned following a random sequence with their collar on the bone level as per clinical inspection and with a standardized inter-implant distance of 5 mm, measured with a metal stent.

Maximum ITV were recorded for each implant at the time of implantation by means of a manual torque wrench (Neodent®, Curitiba - PR, Brazil) to control the level of biomechanical anchorage at the bone-implant interface, not exceeding the maximum value recommended by the manufacturer (60 N.cm). Standard transmucosal healing abutments (3.5 x 4.5 mm, L x Ø) were connected to 20 implants to allow non-submerged healing deprived of actual function, while custom-made abutments (8.0 x 4.5 mm, L x Ø) were connected to other 20 implants to allow for immediate functional loading, as previously described²⁵. The soft tissue was repositioned, appropriately adapted, and sutured with Vicryl 4-0 (Ethicon GmbH, Norderstedt, Germany; Figure 3). Termination was performed after a healing period of 12 weeks following implantation. Bone blocks containing the implants were retrieved and the specimens were processed for non-decalcified histologic and histomorphometric evaluation.

Drilling protocols

Standard and oversized drilling protocols were defined according to the implant diameter and thread dimensions aiming to yield an implant-last drill discrepancy (IDD, the relative dimensional mismatch between the outer implant diameter and the bone wall in the implantation bed) of 0.3 mm and 0.1/0.0 mm, respectively. Standard drilling of the implant socket included a 3-step series of 2-mm pilot, 2-mm twist, and 3.2-mm multi-fluted tapered drills without profiling of the cortical bone (IDD = 0.3 mm in the total 11.5-mm length of the osteotomy; Figure 2a). Oversized instrumentation comprised a 5-step series of 2-mm pilot, 2-mm twist, 3.2-mm and 3.4-mm multi-fluted tapered drills (IDD = 0.1 in the 8-mm central apical length of the osteotomy; Figure 2b) in addition to profiling of the cortical bone by employing a 3.5-mm pilot drill (IDD = 0.0 mm in the 3.5-mm coronal length; Figure 2c).

Analytical methods

The histomorphometrical measurements were done on the two technically best central mesio-distal sections from each specimen, randomly selected, by one blind calibrated examiner (R.N.R.J.). Two rectangular areas of interest (AOI) were determined to represent the central coronal and apical aspects of the implant (7- and 4.5-mm length) due to its dual-tapered body and dynamic thread design (AOI-c and AOI-a, respectively; Figure S1).

The following parameters were measured and calculated:

- 1) The distance from the implant platform to the first coronal mineralized bone-to-implant contact (f-BIC; mm) at the mesial (f-BIC/M) and distal sites (f-BIC/D), representing the

marginal peri-implant bone level (MBL): the height of marginal bone resorption provided that implants were positioned with their collar on the bone level and that occurrence of bone defects (e.g., buccal dehiscence) were adequately recorded.

(2) Bone-to-implant contact (BIC; %) at both AOI (BIC-c and BIC-a) and at the total implant length (BIC-len).

(3) Peri-implant bone density (BD; %): the amount of bone in a specific reference area extending 0.5 mm laterally from the implant encompassing the peri-implant tissues in both AOI-c and AOI-a (BD-c and BD-a, respectively) and on the total implant length (BD-len). Because of the dissimilarities in IDD mainly in the central coronal region, BD-c was analysed within the threads [BD-c/Th] and on the implant perimeter [BD-c/Pe]; the total amount ($BD-c/T = BD-c/Th + BD-c/Pe$) was then calculated.

Statistical analysis

Sample size calculation was based on information of previous study designs. Quantitative data were expressed as mean $\pm$ standard deviation (s.d.) and median. Initially, data were submitted to normality test (Shapiro-Wilk) and equal variance test (Levene). General linear statistical models were then applied for significant differences with the Mann-Whitney test and the independent t-test for nonparametric and parametric data, respectively. Duplicate measurements made on the best section of each sample for BIC-len, within a 1-week interval, were used to test for reproducibility (Table S1). The Spearman's Rank-Order (Rho) Correlation test was taken to evaluate the strength of association between the parameters evaluated herein. The level of significance was set at $P < 0.05$, and the IBM SPSS Statistics software was used (IBM Company Inc., Chicago, IL, USA).

Results

Following completion of the surgical procedures, all animals recovered without any unforeseen incident, and no pre-, intra- and post-operative complications were observed. Insertion torque (N.cm) recorded for each group is presented in Figure 4. Oversized drilling (OD; IDD = 0.1/0.0 mm) produced significantly lower ITV compared to standard drilling of the implant socket (SD; IDD = 0.3 mm, $P = 0.016$).

Overall, histological analysis revealed certain variation in terms of osseointegration and marginal peri-implant bone levels among the groups (Figure 5 and S2). All animals presented a relatively dense bone macrostructure with thick trabecular structures and slender medullary

space, in spite of a minor interindividual variability. Standard drilling of the implant site resulted in distinct resorption of the marginal bone with subsequent crater formation and partial fibrous tissue encapsulation (FTE). One implant undergone total FTE, while another implant was lost – the latter was inserted with the maximum ITV (60 N.cm; Figure 5a and S2a). Implants installed with oversized drilling technique revealed adequate osseointegration and reduced marginal bone changes (Figure 5b and S2b). Only one implant yielded partial osseointegration with extensive bone resorption at the mesial site. Remodeling of the surrounding bone in both central coronal and apical regions of the implants was observed in both groups, characterized by the presence of basic multicellular units (BMUs) and cement lines (Figure 5c-f). However, oversized-drilled sites presented with primary osteonic structures within the threads and overall decreased level of remodeling. Peri-implant regions with low to deficient mineralized tissue exhibited a low to moderate inflammatory infiltrate and bone under active resorption in close proximity to the surface (Figure S3), irrespective of the drilling technique.

The results of the histomorphometric evaluation are presented in Figures 6 and Table 1. Statistical assessment of f-BIC (mm) did not reveal any significant differences between OD and SD, neither at the mesial site nor at the distal site (Figure 6a). However, an increase in CBL could be observed in standard-drilled implant sites with a rather greater intra-group variation compared to oversized-drilled sites. OD showed statistically significant higher values of bone-to-implant contact (%) at both AOI (BIC-c [$P = 0.04$] and BIC-a [$P = 0.012$]), and at the total implant length (BIC-len [$P = 0.005$]) compared to SD (Figure 6b). Bone density values (%) surrounding the central coronal aspect of the impact (AOI-c) are reported in Figure 6c. OD showed statistically significant higher bone density values, within the threads (Th), on the implant perimeter (Pe), and its total amount (T) within the AOI-c (BD-c/Th [$P = 0.01$], BD-c/Pe [$P = 0.03$] and BD-c/T [$P = 0.02$]) compared to SD. %BD values surrounding the apical aspect of the implant (BD-a) and the total implant length (BD-len) are reported in Table 1. Similar amounts of BD-a were observed between the groups, without significant differences. OD showed statistically significant higher BD-len (BD-len/Th [$P = 0.003$], BD-c/Pe [$P = 0.03$] and BD-c/T [$P = 0.01$]) compared to standard drilling.

The Spearman's correlation coefficient (r) between the parameters evaluated herein is described in Table 2. There was a statistically significant, strong negative relationship between ITV and

the following parameters: BIC-c, BD-c/Pe ($P < 0.05$), and BD-c/Th ($P < 0.01$); while, in contrast, BIC-c revealed a strong positive relationship with BD-c/Th and BD-c/Pe ($P < 0.01$).

Discussion

The present study evaluated the effect of an oversized implant site on marginal peri-implant bone level (MBL) and osseointegration of tapered titanium implants, compared to implants inserted with a standard drilling protocol following 12 weeks of non-submerged healing in the minipig mandible. The results obtained herein showed that insertion torque values (ITV) significantly decreased as a function of reducing the implant-last drill discrepancy (IDD; mm). Despite low primary stability, oversized-drilled sites prevented detrimental MBL changes, where healing chambers were initially formed due to sequential tapping and profiling of the bone in respect to the implant macrogeometry. Accordingly, this technique resulted in overall significantly higher percentage bone-to-implant contact (BIC) and peri-implant bone density (BD), characterized by osteonic structures within the threads, confirming the hypothesis. In contrast, higher ITV by employing standard drilling appeared to cause greater marginal bone resorption associated with crater formation and impaired osseointegration.

The level of friction and compressive stress induced at the peri-implant bone is mainly determined by its quality (*i.e.*, aspects of cellular composition and microarchitecture responsible for bone strength) and density (mineral content) in association with aspects of implant macrogeometry and surgical instrumentation. The implant analysed herein presents a full dual tapered body with dynamic progressive threads varying between compressing trapezoidal design on the coronal area to self-tapping triangular threads on the apical part with different sizes. The use of an oversized drilling technique, generating small IDD values, enables formation of healing chambers of varied dimensions. Hence, the final drill employed with a 3.4 mm and 3.5 mm diameter for tapping of the bone site and profiling of the cortex, respectively, resulted in a 0.1 mm narrower implantation bed compared to the implant outer diameter plus a passive cortical layer (3.5 mm in length); *i.e.*, the threads engaged 0.05 mm within the trabecular bone, while there was a passive-fit at the coronal part. Instead, standard drilling technique with a 3.2-mm final drill created a press-fit condition with the dimension of the implantation bed 0.3 mm smaller than the outer implant diameter, while the threads engaged 0.15 mm within the bone, including the cortical layer.

Often conflicting literature data, lack of correlation between ITV and implant stability quotient (ISQ) has previously been reported concerning adapted surgical protocols as it does not depict the quality of the osteotomy itself, degree of bone compression and microdamage²⁶⁻²⁹. Although, a positive correlation has also been shown³⁰⁻³². For instance, undersized and standard drilling techniques (IDD > 0.2 mm), yielding high initial ISQ (> 70%), maintain rather stable values up to 5 weeks of healing, in a similar animal model to that applied herein⁹. In turn, despite oversized drilling (IDD = 0.0 – 0.1 mm) resulting in lower ISQ values ($\leq 60\%$) at the time of implant placement, a large increase in the total implant stability threshold is observed already at the first week up to 3 weeks of healing.

Different levels of implant stability between different osteotomy dimensions is attributed to the effect of lateral mechanical compression to the bone during implant placement. High insertion torque (> 50 N.cm) applied to highly dense and poorly vascularized bone results in increased maximum bone compressive micro strain beyond its threshold (> 3600 $\mu\epsilon$)^{33,34}, increasing the rate of bone remodeling and micromotion despite initial high BIC^{13,29}. Specifically, the microstructural composition of cortical bone is affected by extensive strain, revealing empty osteocytic lacunae associated with multiple bone remodeling units (BMUs)³⁵.

In the present study, despite no significant differences in CBL, histomorphometrically evaluated by means of first BIC, there was a tendency for greater bone resorption in sites drilled with the standard drilling protocol compared to oversized sites. A study evaluating narrower and enlarged sockets (IDD = 0.2 and 0.0 mm, respectively) in a similar animal model has reported comparable CBL of 0.5 mm after 5 weeks *in vivo*, despite occurrence of microcracks in cortical bone in the former group⁹. At the long-term healing of 12 weeks, similar values were also observed in sites receiving only tapping against oversized coronal sites (IDD = 0.15 and -0.05 mm, respectively)¹⁴. However, extensive compression generated in the former group appeared to be more deleterious to the peri-implant bone regeneration under immediate functional loading, leading to formation of crater-like bone defects due to a higher stress concentration³⁶. Conversely, Duyck et al. (2010)¹³ reported significantly higher 4-mm bone loss around implants inserted in press-fit/undersized sites compared to standard drilling with profiling of the cortex (1.3 mm; IDD = 0.4 and 0.0 mm, respectively). However, a recent meta-analysis observed similar CBL between high and low ITV in both human and animal studies³⁷. After 12 weeks of healing, studies show comparably high levels of secondary stability irrespective of the drilling technique and implant macrogeometry.

On the other hand, healing chambers support proliferative mechanisms during osseointegration without prior resorption of necrotic/damaged bone as a result of tension release in the absence of microfractures^{5,9,38}. This event results in extensive, multidirectional woven bone formation on the implant surface and from the surgically instrumented pristine bone walls towards the central region of the chamber¹¹. Nonetheless, much of the available literature on oversized drilling has shown similar BIC after 3 weeks of healing in comparison to standard and/or undersized surgical protocols. In turn, percentage of bone density has been shown to be similar among different instrumentation techniques only between 3 and 4 weeks²⁰, with oversized-drilled sites resulting in rather lower values. Accordingly, fast bone matrix apposition and mineralization in healing chambers may develop similar amount of bone density and, therefore, implant stability compared to that found when implants undergo interfacial bone remodeling in narrower sites.

There are several possible explanations to the lack of corresponding data to the present findings, such as the application of different animal models and, consequently, varied alveolar contour, thickness and quality, in addition to modifications in implant surface properties and macrogeometry. The majority of the studies evaluating oversized surgical protocols apply moderately rough surfaces with another chemical modification than that applied herein, possibly revealing different levels of surface energy, hydrophilicity and chemical composition. In the present study, implants with a hydrophilic surface, proven to positively modulate the early cellular response and accelerate bone formation, were used^{39,40}. Although hydrophobic surfaces are shown to achieve similar amounts of osseointegration^{41,42}, healing chambers are suggested to support the osteoconductive properties of novel bioactive surfaces, further lessening the healing period⁴³.

Furthermore, implant macrogeometry yield different stress distribution to the bone. Implants presenting a tapered body have been shown to achieve better primary stability when compared to cylindrical implants⁴⁴. In respect to thread design, whilst square-shaped reveals higher potential for bone compression, trapezoidal and triangular threads facilitate surgical placement^{45,46}. In this sense, some studies have demonstrated the benefit of the hybrid healing pathway to enhance osseointegration and load-bearing capacity of implants placed in over dimensioned sites, which integrates both interfacial remodelling and intramembranous-like healing mode^{20,21}. Thus, a dynamic thread design similar to that used herein may contribute to

maintain proper levels of primary implant stability in over dimensioned high-density bone sites, while allowing a rapid onset and establishment of osseointegration.

In context, minipigs are often used as an animal model to evaluate the potential of implants to osseointegrate because of the comparable bone metabolic rate (ratio of 1:2) and heterodont dentition to that in humans⁴⁷. Nonetheless, the generalizability of these findings is subject to certain limitations. The edentulous alveolar crest of minipigs present a rather thick cortical bone and moderate to dense trabeculae with high biomechanical properties, which might synergistically intensify the effect of lateral compression as less porous, non-elastic and poorly vascularized bone is more inclined towards microstructural stresses, compromising normal metabolic activity and tissue regeneration⁴⁸. In addition, narrow residual alveolar ridges are frequently found, which represent a high risk of developing peri-implant bone dehiscence and bone resorption⁴⁹. Thus, MBL and parameters of osseointegration in standard-drilled sites presented herein should be interpreted with precaution, since the experimental conditions do not represent challenging cases wherein this technique is often utilized (*e.g.*, immediate loading in thin crestal cortical bone and/or low-density cancellous bone).

Conclusion

Within the limitations of the present study, oversized surgical preparation of the implant site yielded low insertion torque of tapered implants with a progressive thread geometry, but enhanced osseointegration and peri-implant bone density, and preserved better the MBL in the mandible of minipigs. Reduced lateral compression of high-density trabecular bone with a thick cortical layer appears to preserve better the marginal peri-implant bone level.

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Conflict of interest

Rainde N. R. de Jesus received the experimental titanium disks from Neodent® (Curitiba-PR, Brazil; process no. PAP 1204-17) to support this study, as part of her doctoral research. None of the co-authors have any other conflict of interest in regards to this study.

References

1. Javed F, Ahmed HB, Crespi R, Romanos GE. Role of primary stability for successful osseointegration of dental implants: Factors of influence and evaluation. *Interv Med Appl Sci.* 2013;5(4):162-167.
2. Lioubavina-Hack N, Lang NP, Karring T. Significance of primary stability for osseointegration of dental implants. *Clin Oral Implants Res.* 2006;17(3):244-250.
3. Ribeiro-Rotta RF, de Oliveira RC, Dias DR, Lindh C, Leles CR. Bone tissue microarchitectural characteristics at dental implant sites part 2: correlation with bone classification and primary stability. *Clin Oral Implants Res.* 2014;25(2):47.
4. Coelho PG, Marin C, Teixeira HS, et al. Biomechanical evaluation of undersized drilling on implant biomechanical stability at early implantation times. *J Oral Maxillofac Surg.* 2013;71(2):69.
5. Baires-Campos, Jimbo R, Bonfante EA, et al. Drilling dimension effects in early stages of osseointegration and implant stability in a canine model. *Med Oral Patol Oral Cir Bucal.* 2015;20(4):471.
6. Berglundh T, Abrahamsson I, Lang NP, Lindhe J. De novo alveolar bone formation adjacent to endosseous implants. *Clin Oral Implants Res.* 2003;14(3):251-262.
7. Gomes JB, Campos FE, Marin C, et al. Implant biomechanical stability variation at early implantation times in vivo: an experimental study in dogs. *Int J Oral Maxillofac Implants.* 2013;28(3):128.
8. Friberg B, Sennerby L, Gröndahl K, Bergström C, Bäck T, Lekholm U. On cutting torque measurements during implant placement: a 3-year clinical prospective study. *Clinical implant dentistry and related research.* 1999;1(2):75-83.
9. Eom T, Kim H, Jeon G, Yun M, Huh J, Jeong C. Effects of Different Implant Osteotomy Preparation Sizes on Implant Stability and Bone Response in the Minipig Mandible. *Int J Oral Maxillofac Implants.* 2016;31(5):997-1006.
10. Stoccherio, Toia M, Jinno Y, et al. Influence of different drilling preparation on cortical bone: A biomechanical, histological, and micro-CT study on sheep. *Clin Oral Implants Res.* 2018.
11. Coelho PG, Suzuki M, Guimaraes MVM, et al. Early bone healing around different implant bulk designs and surgical techniques: A study in dogs. *Clin Implant Dent Relat Res.* 2010;12(3):202-208.

12. Jimbo R, Tovar N, Yoo DY, Janal MN, Anchieta RB, Coelho PG. The effect of different surgical drilling procedures on full laser-etched microgrooves surface-treated implants: an experimental study in sheep. *Clin Oral Implants Res.* 2014;25(9):1072-1077.
13. Duyck J, Corpas L, Vermeiren S, et al. Histological, histomorphometrical, and radiological evaluation of an experimental implant design with a high insertion torque. *Clin Oral Implants Res.* 2010;21(8):877-884.
14. Stavropoulos A, Cochran D, Obrecht M, Pippenger BE, Dard M. Effect of Osteotomy Preparation on Osseointegration of Immediately Loaded, Tapered Dental Implants. *Adv Dent Res.* 2016;28(1):34-41.
15. Stocchero M, Toia M, Jinno Y, et al. Influence of different drilling preparation on cortical bone: A biomechanical, histological, and micro-CT study on sheep. *Clin Oral Implants Res.* 2018.
16. Campos, Gomes JB, Marin C, et al. Effect of drilling dimension on implant placement torque and early osseointegration stages: an experimental study in dogs. *J Oral Maxillofac Surg.* 2012;70(1):43.
17. Coelho PG, Suzuki M, Guimaraes MV, et al. Early bone healing around different implant bulk designs and surgical techniques: A study in dogs. *Clinical Implant Dentistry and Related Research.* 2010;12(3):202-208.
18. Duyck J, Roesems R, Cardoso MV, Ogawa T, De Villa Camargos G, Vandamme K. Effect of insertion torque on titanium implant osseointegration: an animal experimental study. *Clin Oral Implants Res.* 2015;26(2):191-196.
19. Galli S, Jimbo R, Tovar N, et al. The effect of osteotomy dimension on osseointegration to resorbable media-treated implants: a study in the sheep. *J Biomater Appl.* 2015;29(8):1068-1074.
20. Jung U, Kim S, Kim Y, Cha J, Lee I, Choi S. Osseointegration of dental implants installed without mechanical engagement: a histometric analysis in dogs. *Clin Oral Implants Res.* 2012;23(11):1297-1301.
21. Kim M, Jung U, Kim S, Lee J, Lee I, Choi S. Bone apposition on implants coated with calcium phosphate by ion beam assisted deposition in oversized drilled sockets: a histologic and histometric analysis in dogs. *Journal of Periodontal and Implant Science.* 2013;43(1):18-23.
22. Rea M, Lang NP, Ricci S, Mintrone F, González González G, Botticelli D. Healing of implants installed in over- or under-prepared sites--an experimental study in dogs. *Clin Oral Implants Res.* 2015;26(4):442-446.

23. Berglundh T, Stavropoulos A. Preclinical in vivo research in implant dentistry. Consensus of the eighth European workshop on periodontology. *J Clin Periodontol.* 2012;39 Suppl 12:1-5.
24. Sartoretto SC, Alves, Adriana Terezinha Neves Novellino, Resende RFB, Calasans-Maia J, Granjeiro JM, Calasans-Maia MD. Early osseointegration driven by the surface chemistry and wettability of dental implants. *Journal of Applied Oral Science.* 2015;23(3):279-287.
25. Elian N, Bloom M, Dard M, Cho S, Trushkowsky RD, Tarnow D. Radiological and micro-computed tomography analysis of the bone at dental implants inserted 2, 3 and 4 mm apart in a minipig model with platform switching incorporated. *Clin Oral Implants Res.* 2014;25(2).
26. Akça K, Kökat AM, Cömert A, Akkocaoğlu M, Tekdemir I, Cehreli MC. Torque-fitting and resonance frequency analyses of implants in conventional sockets versus controlled bone defects in vitro. *Int J Oral Maxillofac Surg.* 2010;39(2):169-173.
27. Degidi M, Dapriale G, Piattelli A. Influence of underpreparation on primary stability of implants inserted in poor quality bone sites: an in vitro study. *J Oral Maxillofac Surg.* 2015;73(6):1084-1088.
28. Degidi M, Perrotti V, Strocchi R, Piattelli A, Iezzi G. Is insertion torque correlated to bone-implant contact percentage in the early healing period? A histological and histomorphometrical evaluation of 17 human-retrieved dental implants. *Clin Oral Implants Res.* 2009;20(8):778-781.
29. Freitas AC, Bonfante EA, Giro G, Janal MN, Coelho PG. The effect of implant design on insertion torque and immediate micromotion. *Clin Oral Implants Res.* 2012;23(1):113-118.
30. Consolo U, Travaglini D, Todisco M, Trisi P, Galli S. Histologic and biomechanical evaluation of the effects of implant insertion torque on peri-implant bone healing. *J Craniofac Surg.* 2013;24(3):860-865.
31. Halldin A, Jimbo R, Johansson CB, et al. Implant stability and bone remodeling after 3 and 13 days of implantation with an initial static strain. *Clin Implant Dent Relat Res.* 2014;16(3):383-393.
32. Halldin A, Jimbo R, Johansson CB, et al. The effect of static bone strain on implant stability and bone remodeling. *Bone.* 2011;49(4):783-789.
33. Hudieb M, Wakabayashi N, Suzuki T, Kasugai S. Morphologic classification and stress analysis of the mandibular bone in the premolar region for implant placement. *Int J Oral Maxillofac Implants.* 2010;25(3):482-490.

34. Sugiura T, Horiuchi K, Sugimura M, Tsutsumi S. Evaluation of threshold stress for bone resorption around screws based on in vivo strain measurement of miniplate. *J Musculoskelet Neuronal Interact.* 2000;1(2):165-170.
35. Slaets E, Carmeliet G, Naert I, Duyck J. Early cellular responses in cortical bone healing around unloaded titanium implants: an animal study. *J Periodontol.* 2006;77(6):1015-1024.
36. Duyck J, Rønold HJ, Van Oosterwyck H, Naert I, Vander Sloten J, Ellingsen JE. The influence of static and dynamic loading on marginal bone reactions around osseointegrated implants: an animal experimental study. *Clin Oral Implants Res.* 2001;12(3):207-218.
37. Berardini M, Trisi P, Sinjari B, Rutjes AWS, Caputi S. The effects of high insertion torque versus low insertion torque on marginal bone resorption and implant failure rates: a systematic review with meta-analyses. *Implant Dent.* 2016;25(4):532-540.
38. Berglundh, Abrahamsson I, Lang NP, Lindhe J. De novo alveolar bone formation adjacent to endosseous implants: A model study in the dog. *Clinical Oral Implants Research.* 2003;14(3):251-262.
39. Shanbhag S, Shanbhag V, Stavropoulos A. Genomic analyses of early peri-implant bone healing in humans: a systematic review. *Int J Implant Dent.* 2015;1(1):5.
40. Lotz EM, Berger MB, Schwartz Z, Boyan BD. Regulation of osteoclasts by osteoblast lineage cells depends on titanium implant surface properties. *Acta Biomater.* 2018;68:296-307.
41. de Jesus, Stavropoulos A, Oliveira MTF, Soares PBF, Moura CCG, Zanetta-Barbosa D. Histomorphometric evaluation of a dual acid-etched vs. a chemically modified hydrophilic dual acid-etched implant surface. An experimental study in dogs. *Clinical Oral Implants Research.* 2017;28(5):551-557.
42. de Jesus, Carrilho E, Antunes PV, et al. Interfacial biomechanical properties of a dual acid-etched versus a chemically modified hydrophilic dual acid-etched implant surface: an experimental study in Beagles. *Int J Implant Dent.* 2018;4(1):28.
43. Coelho PG, Jimbo R. Osseointegration of metallic devices: Current trends based on implant hardware design. *Archives of Biochemistry and Biophysics.* 2014;561:99-108.
44. Alves CC, Neves M. Tapered implants: from indications to advantages. *Int J Periodontics Restorative Dent.* 2009;29(2):161-167.
45. Chang P, Chen Y, Huang C, Lu W, Chen Y, Tsai H. Distribution of micromotion in implants and alveolar bone with different thread profiles in immediate loading: a finite element study. *Int J Oral Maxillofac Implants.* 2012;27(6):96.

46. Lima de Andrade C, Carvalho MA, Bordin D, da Silva WJ, Del Bel Cury, Altair Antoninha, Sotto-Maior BS. Biomechanical Behavior of the Dental Implant Macrodesign. *Int J Oral Maxillofac Implants*. 2017;32(2):264-270.
47. Stadlinger B, Pourmand P, Locher MC, Schulz MC. Systematic review of animal models for the study of implant integration, assessing the influence of material, surface and design. *J Clin Periodontol*. 2012;39 Suppl 12:28-36.
48. Simons W, De Smit M, Duyck J, Coucke W, Quirynen M. The proportion of cancellous bone as predictive factor for early marginal bone loss around implants in the posterior part of the mandible. *Clin Oral Implants Res*. 2015;26(9):1051-1059.
49. Qahash M, Susin C, Polimeni G, Hall J, Wikesjö UME. Bone healing dynamics at buccal peri-implant sites. *Clin Oral Implants Res*. 2008;19(2):166-172.

Tables

TABLE 1 Total amount of bone density (BD-T, %) within the AOI-a (BD-a) and surrounding the total implant length (BD-len) for both groups

Parameter	Standard drilling (SD)		Oversized drilling (OD)		P value
	Mean $\pm$ s.d.	Median	Mean $\pm$ s.d.	Median	
BD-a/T	44.47 $\pm$ 31.49	52.05	71.94 $\pm$ 9.66	72.45	0.11
BD-len/T	25.32 $\pm$ 17.11	31.86	54.46 $\pm$ 10.72	50.51	0.01

Bold numbers indicate statistically significant differences.

TABLE 2 The Spearman's correlation coefficient (r) values between parameters for the surgical drilling techniques (n = 9; 2-tailed)

		ITV	f-BIC/M	f-BIC/D	BIC-c	BIC-a	BD-c/Th	BD-c/Pe
ITV	r	1	0.394	0.583	(-) .692*	(-) .622	(-) .815**	(-) .742*
	Sig.		0.294	0.1	0.039	0.073	0.007	0.022
BIC-c	r	(-) .692*	(-) .181	(-) .248	1	0.665	.946**	.866**
	Sig.	0.039	0.642	0.521		0.051	0	0.003
BIC-a	r	(-) .622	(-) .063	(-) .034	0.665	1	.670*	0.48
	Sig.	0.073	0.872	0.932	0.051		0.048	0.191
BD-c/T	r	(-) .776*	(-) .259	(-) .215	.898**	0.524	.942**	.996**
	Sig.	0.014	0.501	0.578	0.001	0.147	0	0
BD-a/T	r	(-) .369	0.33	0.32	0.587	.787*	0.547	0.527
	Sig.	0.328	0.386	0.401	0.097	0.012	0.127	0.145

Bold numbers indicate statistically significant differences. Correlation is significant at the 0.05 (*) and 0.01 level (**), respectively.

Figures

Figure 1. Schematic illustration of the threads' geometric profile [shape, width, depth, pitch (mm) and face angle] and SEM micrographs of the surface microtopography of the experimental implants (Neodent®, Curitiba - PR, Brazil). (a) Helix GM® (HLX, 11.5 mm x 3.5 mm, L x Ø; n = 20) is a full dual tapered implant containing an active apex and helicoidal flutes. This implant presents a dynamic progressive thread geometry with double threads varying between compressing square and trapezoidal on the coronal area, reverse buttress on the central aspect, and self-tapping triangular threads (V-shaped) on the apical part of the implant with varied dimensions finishing in 0.55 mm. Due to its potential for either bone compression or cutting, HLX is indicated for all types of bone density and immediate implant placement post extraction. HLX presents platform switching and the Grand Morse™ (GM) implant-abutment connection interface characterized by a deep 16° morse taper connection. (b) All implants present surface hydrophilicity (marketed as Acqua™; SAE-HD) with moderately rough microtopography.

Figure 2. Schematic presentation of the study design including the various steps of the different protocols for the surgical preparation of the implant site in relation to the last drill diameter [standard drilling (SD) vs. oversized drilling (OD)]. (a) SD included a 3-step series of 2-mm pilot, 2-mm twist, and 3.2-mm multi-fluted tapered drills without profiling of the cortical bone (IDD = 0.3 mm in the total 11.5-mm length of the osteotomy). (b) OD comprised a 5-step series of 2-mm pilot, 2-mm twist, 3.2-mm and 3.4-mm multi-fluted tapered drills (IDD = 0.1 in the 8-mm central apical length of the osteotomy). (c) In addition, profiling of the cortical bone was

Figure 3. Four implants were inserted in each side of the mandible (n = 8 implants/animal). Implants were positioned following a random sequence with their collar on the bone level with a standardized inter-implant distance of 5 mm. Standard transmucosal healing abutments (3.5 x 4.5 mm, L x Ø) were connected to 20 implants to allow non-submerged healing deprived of actual function (from which a total of 10 implants were analysed in the present study), while custom-made abutments (8.0 x 4.5 mm, L x Ø) were connected to other 20 implants to allow for immediate functional loading. (b) The soft tissue was repositioned, appropriately adapted, and sutured with Vicryl 4-0.

Figure 4. Mean $\pm$ s.d. and box plots of the data on insertion torque values (ITV; N.cm) representing the interquartile range (IQR; box) between the lower and upper quartile, median (white line), minimum and maximum values (horizontal lines outside the IQR). Oversized drilling (OD) produced significantly lower ITV compared to standard drilling of the implant socket (SD; $P < 0.016$).

Figure 5. Representative microphotographs illustrating the qualitative histology for both groups, comprising standard drilling (SD) and oversized drilling (OD) of the implant site after 12 weeks in vivo. (a) SD resulted in distinct resorption of the marginal bone with subsequent crater formation and partial fibrous tissue encapsulation. (b) OD resulted in adequate osseointegration and reduced marginal bone changes. (c-d) Remodeling of the surrounding bone in both central coronal and apical regions of the implants was observed in both SD and OD groups, characterized by the presence of basic multicellular units (BMUs) and cement lines. (e-f) Peri-implant regions with low to deficient mineralized tissue exhibited a low to moderate inflammatory infiltrate. Red dashed rectangle, magnified area. Green asterisks, BMUs. Black arrowheads, osteoclasts. Black arrows, bone resorption. Red arrowheads, osteoblasts. Red asterisks, bone formation. White arrowheads, cement lines. White asterisks, osteonic structures. Yellow dotted circumference and asterisks, inflammatory infiltrate. Overview magnification x20. Toluidine blue stain.

Figure 6. Mean $\pm$ s.d. of the data on (a) first bone-to-implant contact (f-BIC; mm), (b) BIC (%) within both AOI, coronal and apical, and at the total implant length, and (c) peri-implant bone density (BD; %) within the AOI-c within the threads, on the implant perimeter, and its total amount. f-BIC did not reveal any significant differences between oversized and standard drilling. For all parameters of BIC and BD, oversized drilling resulted in statistically significant higher values compared to standard drilling.

Supplementary information

Material and methods

Implant system

This implant presents a dynamic progressive thread geometry with double threads varying between compressing square and trapezoidal on the coronal area, reverse buttress on the central aspect, and self-tapping triangular threads (V-shaped) on the apical part of the implant with varied dimensions finishing in 0.55 mm. Due to its potential for either bone compression or cutting, HLX is indicated for all types of bone density and immediate implant placement post extraction. HLX presents platform switching and the Grand Morse™ (GM) implant-abutment connection interface characterized by a deep 16° morse taper connection.

Proprietary technology treatment

All implants present surface hydrophilicity (marketed as Acqua™; SAE-HD) with moderately rough microtopography generated by means of sandblasting with 1.4 – 1.8 µm abrasive particles and dual acid-etching with hydrochloric (HCl) and sulfuric acid (H₂SO₄) (Figure 1b). In addition, the implants receive microwave treatment under nitrogen protection to prevent air exposure, and insertion in 0.9% isotonic saline (NaCl) solution resulting in significant hydrophilicity. The surface exhibits the following 3D surface roughness parameters: S_a: 1.26 µm ± 0.17; S_z: 16.20 µm ± 7.8; S_{dr}: 1.21%; S_{ds}: 643.33 1/mm² ± 37.74; S_{sk}: -0.43.

Animal housing and husbandry

Approximately 2 weeks previous to the experimental procedures, all animals were group-housed in standard boxes (4 mm²) with long straw as bedding material to satisfy rooting behaviour and adapt to the research facility. Throughout the trial, the minipigs were kept in a temperature-controlled environment (18 ± 1 °C) under relative humidity between 45 and 90% and 12-h circadian rhythm. Environmental enrichment was provided with daily interactions with soft rubber cones and bucket in chain. The diet consisted of a restricted soft commercial food to control their weight gain and water *ad libitum*. The level of pain, distress, and suffering was assessed as welfare indicators by blind caretakers and a humane endpoint was established prior to commencement of the experiment (Ellegaard et al., 2010).

Anaesthesia protocol

Briefly, sedation of the minipigs was achieved with an i.m. injection of dexmedetomidine (10 µg/kg; DexDomitor®, Orion Corporation, Espoo, Finland), butorphanol (0.1 mg/kg; Dolorex®, MSD Animal Health, Wellington, New Zealand), midazolam (0.5 mg/kg; Dormipnol®, VioFar LTD, Acharnes, Greece) and ketamine chlorate (3 mg/kg; Ketamidol®, Richter Pharma AG, Wels, Austria). General anesthesia was induced with an intravenous infusion of propofol (0.5 - 1.5 mg/kg; Propofol®, MCT/LCT Fresenius, Fresenius Kabi, Uppsala, Sweden) followed by isoflurane (1 – 2%; Iso-vet®, Piramal Healthcare UK Limited, Morpeth, UK) and maintained in oxygen through an endotracheal tube. Intraoperative local anesthesia of xylocaine with adrenalin (20 mg/ml + 12.5 mg/ml, 3.6 ml each side; Xylocaine® adrenalin, Astra AB, Södertälje, Sweden) was given to reduce the dosage of systemic analgesic, as well as to increase hemostasis and provide immediate postoperative pain control. The animals were treated for analgesia with fentanyl CRI (0.05 µg/kg/h; Fentanyl/Janssen, Janssen, Cilag AEBE, Greece) and meloxicam (0.4 mg/kg/d; Loxicom, Norbrook Laboratories, UK) for 3 days. The subsequent healing period progressed uneventfully. Termination was performed by inducing cardiopulmonary arrest with an intra-cardiac injection of anaesthetic overdose of a 20% solution of pentobarbital (60 mg/ml; Pentobarbitalnatrium, Apoteket AB, Sweden) and, upon removal of the mandibles and retrieval of bone blocks, some implant sites showed remarkable bone loss.

Teeth extraction

All mandibular premolars (P1 – P4) and the first molar (M1) were extracted bilaterally following segmentation of the teeth by means of a diamond bur on a high speed hand-piece with internal irrigation. Intraoperative digital periapical radiographs were taken to control the absence of root remnants. The edentulous ridges were allowed to heal for 12 weeks.

Histotechnical procedure

Bone blocks containing the implants were retrieved, overlying soft tissues were removed and the specimens were fixed by immersion in 10% buffered formalin solution for 2 weeks. The fixed specimens were rinsed in running water for 24 h, gradually dehydrated in a series of ascending concentrations of alcohol and embedded in methyl-methacrylate based resin (Technovit® 7200, VLC, Hereaus Kulzer®, Hanau, Germany). Subsequently, the samples were photopolymerized and hard blocks were obtained. Two to three central longitudinal sections, approximately 300 µm thick, were cut in a mesio-distal direction and parallel to the long axis of the implant in an EXAKT thin section cutting technology (EXAKT Advanced Technologies

GmbH, Norderstedt, Germany). Cutting plane definition was enabled by examination of digital peri-apical radiographs of the embedded hard blocks. Thereafter, the ground sections were grounded and polished to a final thickness of 25 µm on average following previously described protocols (Donath & Breuner 1982) using a desktop grinding-polishing unit (EXAKT). Finally, the sections were stained with toluidine blue (Histolab Products AB, Västra Frölunda, Sweden) and imaged at a desktop digital slide scanner (NanoZoomer-SQ Digital Pathology System, C13140-01, Hamamatsu®, Hamamatsu City, Japan) connected to a computer with dedicated imaging software to create whole images of the biopsy (NDP.serve and NDP.view, Hamamatsu®).

Supplementary table

TABLE S1 Results of the reproducibility evaluation showed no significant differences ($P > 0.05$) between the 2 sets of measurements regarding bone-to-implant contact (BIC; %) in the total implant length (BIC-len; %) evaluated with the independent t-test

	Standard drilling (SD)			Oversized drilling (OD)		
	1st measurement	2nd measurement	<i>P</i> value	1st measurement	2nd measurement	<i>P</i> value
BIC-len	0.00	0.00		31.76	31.68	
	5.62	5.08		17.78	17.27	
	11.42	11.18		25.62	25.30	
	18.14	18.06		27.22	26.99	
	--	--		32.23	31.89	
Mean ± s.d.	8.79 ± 7.78	8.58 ± 7.80	0.97	26.92 ± 5.85	26.62 ± 5.97	0.93

Supplementary figures

Figure S1. Representative microphotographs illustrating the methodology applied to the histomorphometrical analysis. (a) The sections were divided into two rectangular areas of interest (AOI) to represent the central coronal and apical aspects of the implant (7- and 4.5-mm length) due to its dual-tapered body and dynamic thread design (AOI-c and AOI-a, respectively; red dashed lines). The distance from the implant platform to the first mineralized bone-to-implant contact (f-BIC, mm; blue lines) at the mesial (f-BIC/M) and distal sites (f-BIC/D), represents the marginal peri-implant bone level (MBL), considering that the implants were positioned with their collar on the bone level. (b-d) Images at a higher magnification illustrate the bone-to-implant contact (BIC, %; yellow lines) and the peri-implant bone density (BD; %) 0.5 mm laterally from the implant encompassing the peri-implant tissues in both (b and c) AOI-c and (d) AOI-a and on the total implant length (BD-len). Because of the dissimilarities in IDD mainly in the central coronal region, BD-c was analysed within the threads [BD-c/Th] and on the implant perimeter [BD-c/Pe]); the total amount ($BD-c/T = BD-c/Th + BD-c/Pe$) was then calculated. Colour code: (b and d) total amount of BD (T; purple); (c) BD within the threads (Th; red); and BD on the implant perimeter (Pe; blue). Overview magnification x20. Toluidine blue stain. Scale bar: (a) 1 mm and (b-d) 250 μm.

(a) Standard drilling (SD)

(b) Oversized drilling (OD)

Figure S2. Representative microphotographs from all implants at 12 weeks of healing, comprising (a) standard and (b) oversized drilling of the implant site. One implant installed with the SD protocol undergone total FTE possibly due to the close proximity to the remaining canine root (< 1 mm), while another implant was lost (implant filled in black) the latter was inserted with the highest ITV (60 N.cm). Overview magnification x20. Toluidine blue stain. Scale bar: 1 mm.

Figure S3. Peri-implant regions with low to deficient mineralized tissue exhibited a low to moderate inflammatory infiltrate and bone under active resorption (black arrows) in close proximity to the implant surface characterized by the presence of multinucleated cells (orange arrowheads), irrespective of the drilling technique. Toluidine blue stain. Scale bar: 50 μ m.

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