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Citation for the published paper:

Galli, Silvia; Jimbo, Ryo; Tovar, Nick; Yoo, Daniel; Anchieta, Rodolfo; Yamaguchi, Satoshi; Coelho, Paulo G. (2015). The effect of osteotomy dimension on osseointegration to resorbable media-treated implants : a study in the sheep. Journal of Biomaterials Applications, vol. 29, issue 8, p. null

URL: <https://doi.org/10.1177/0885328214553958>

Publisher: Sage

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The effect of osteotomy dimension on osseointegration to resorbable media treated implants: a study in the sheep.

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Running head: Ideal osteotomy for resorbable media blasted surface

Key words: implant surface; resorbable surface – blasted surface; removal torque; histology; osseointegration.

Abstract

The drilling technique and the surface characteristics are known to influence the healing times of oral implants. The influence of osteotomy dimension on osseointegration of microroughened implant surfaces treated with resorbable media blasting (RBM) was tested in an *in vivo* model.

Ninety-six implants (ø4.5 mm, 8 mm in length) with RBM treated surfaces were placed in the ileum of six sheep. The final osteotomy diameters were 4.6 mm (Reamer), 4.1 mm (Loose) 3.7 mm (Medium) and 3.2 mm (Tight). After 3 and 6 weeks healing the implants were biomechanically tested and histologically evaluated. Statistical analysis was performed using Page L trend test for ordered and paired sample and linear regression, with significance level at $p < 0.05$.

An overall increase in all dependent variables was observed with the reduction of osteotomy diameter. In addition, all osseointegration scores increased over time. At 3 weeks, the retention was significantly higher for smaller osteotomies. The histological sections depicted intimate contact of bone with all the implant surfaces and osteoblast lines were visible in all sections.

The RBM microroughened surfaces achieved successful osseointegration for all the instrumentation procedures tested, with higher osseointegration scores for the press-fit insertion group.

Key words: implant surface; resorbable surface – blasted surface ; removal torque; histology; osseointegration.

1. Introduction

Implant surface has long been identified as one of the crucial factor for successful osseointegration¹⁻³. Surface modifications plays an important role in the early phases of implant healing, when osteogenic cells are recruited and migrate directly onto the implant surface. It has been demonstrated that the physical and chemical properties of titanium surfaces modulate platelet activation, growth factor release and the blood cloth stabilization due to a direct interaction of fibrin fibers with the implant interface⁴.

Roughened surfaces were found to provide better conditions for the cell attachment and cell spreading due to their increased surface area⁵ and, in addition, these have been shown to influence cell proliferation and differentiation^{6, 7}. Accordingly, numerous *in vivo* studies presented a stronger bone response to rougher surfaces compared to turned surfaces⁸⁻¹⁰. Similar evidences were found clinically, in which implants with half turned and half treated surface were placed in humans¹¹. When the implants were retrieved for histological analysis after 6 months, the implant portion with the roughened surface presented a significantly higher bone-to-implant contact than the smooth surface¹¹.

Besides surface topography, surface chemistry has been modified by incorporation of different bioactive substances, such as calcium, magnesium, phosphates or fluorides, to further promote bone regeneration, proteins adsorption and cell attachment on the implants¹²⁻¹⁶. One method to modify the texture of titanium surfaces while depositing hydroxyapatite or calcium phosphate (CaP) on them, is blasting with resorbable biocompatible bioceramics (resorbable blasting media or RBM)¹⁷⁻²⁰(Coelho and Lemon 2009, Marin et al 2008 and 2010). The bioceramic

blasting particles produce a moderately rough topography and they can be incorporated into the titanium surfaces and be released in bone after implant placement, which has been suggested to increase the bioactivity.

Extensive surface characterization has shown that RBM-treated surfaces are less rough than titanium surfaces blasted with alumina particles and possess a non-homogenous distribution of CaP, depending on several factors, such as the composition of the blasting media, the particle size and other processing parameters²¹. This incorporation technique overcomes the drawbacks of the thick plasma-sprayed HA and CaP coatings, which exhibited a low clinical survival in the long-term due to debonding of the coating at the implant interface^{22, 23}. It has been reported that RBM obtained a higher and faster biomechanical fixation, after placement in dogs^{24, 25} and newly formed bone was observed in close contact with the surfaces²⁴.

A great amount of experiments focused on the impact of the different surface modifications on osseointegration, while a significantly smaller body of literature exists regarding the influence of the drilling protocol in the host response to specific surfaces. Osteotomy preparation, in combination with implant macrogeometry and the quality of the implant bed, determines the initial mechanical interaction between the implant and the bone (primary stability) and it is one of the factors that ultimately determine the biological stabilization of the implant²⁶⁻²⁸.

Testing the outcome of implants with bioactive surfaces in sites prepared with different surgical procedures would be of great interest if one is attempting to maximize osseointegration at the shortest period of time possible. Thus, the objective of the current investigation was to compare the bone healing around resorbable blasting media treated implants, placed under different drilling protocols in a sheep model, by means of biomechanical and histological testing.

2. Material and Methods

Implants and surgical procedures

Implants treated with resorbable media blasting were used in this study (BioHorizon, 4.5 mm in diameter by 8 mm length).

The implants were placed in 6 Finnish Dorset cross-breed sheep, each one weighting approximately 150 pounds and being 1.5 years old, under the ethical permit released by the Ethics Committee for Animal Research at the École Nationale Vétérinaire d'Alfort (Maisons-Alfort, Val-de-Marne, France). The sheep were acclimatized 2 weeks in the facility before the beginning of the experimental procedures.

At day 0 of the experiment, the sheep were anesthetized with Sodium Pentothal (15-20 mg/kg) in Normasol solution in the jugular vein. The hips were shaved, to expose the skin of surgical site for the implant placement. The area was disinfected with Povidine solution and then delimited by aseptic drapes. The animals were constantly monitored for vital parameters during the surgical procedure, by an automated system that provided ventilation and sustain of the anesthesia with Isoforane (1.5-3%) in O₂/N₂O (50/50). Antibiotic (500 mg Cefazolina) was administered pre-operatively intravenously.

The surgery started with skin incision and muscles and the iliac crest was exposed by blunt dissection. Following 4 different drill sequences, four osteotomies were prepared, so to obtain implants sites with different final diameters: Reamer (R)

4.6 mm (final instrumentation hand reamer), Loose (L) 4.1 mm, Medium (M) 3.7 mm and Tight (T) 3.2 mm. Each sheep received 4 implants on one side at day 0 of experiment and 4 implants on the other side 3 weeks after the first surgery. A total of 48 implants were placed, corresponding to 4 experimental groups (n=6 per healing time).

Removal torque and histomorphometry

Six week after the first surgery (day 0), the animals were euthanized with an overdose of Sodium Pentothal and the hips were dissected. Half of the implants had been in situ for 3 weeks and the other half for 6 weeks. Twenty-four implants undergone biomechanical test by means of removal torque (Tonhichi, torque gauge wrench) while the other twenty-four implants were harvested with the surrounding bone and were processed for non-decalcified histology. The bone blocks were embedded in resin (methylmethacrylate, MMA) after dehydration in increasing ethanol concentrations. Sections were cut and grinded down to thin slices, following the cutting-grinding standard procedure²⁹,

After staining with toluidine blue dye, the samples were observed using the optical microscopy with magnifications from $\times 50$ to $\times 200$ (Leica DM2500M, Leica Microsystems GmbH, Wetzlar, Germany). The bone-to-implant contact (BIC) and the bone area fraction occupancy (BAFO) within the implant threads were calculated by means of specialized computer software (Leica Application Suite, Leica Microsystems GmbH, Wetzlar, Germany)..

Statistics

To evaluate the effects of drilling techniques on the osseointegration parameters at two healing times the Page-L trend test for related and ordered samples was used. This test is appropriate when it is reasonable to predict an order of the treatment effects, as it applies to the current case, and when the samples are related (different samples in the same animal). The null hypothesis was that the scores of the different instrumentation techniques were equals . To evaluate the effects of instrumentation over time linear regression analysis was used. Statistical significance was set at 5% ($\alpha = 0.05$).

3. Results

Animals

All the animals recovered well from the surgery and showed no signs of complications, infection or disease during the whole observation period. All the implants were stable when examined immediately after euthanasia; therefore no implant was excluded from the study.

Removal Torque

The removal torque (RTQ) values and the related descriptive statistics are presented in Table 1 and Figure 1. In general, an overall increase in removal torque values was observed between 3 and 6 weeks.

The Page trend statistical test showed a significant trend in RTQ values among the different instrumentation procedures, ordered from the widest to the tightest osteotomy ($p < 0.05$). At 3 weeks of healing the RTQ values were statistically significantly ordered, with higher torques correlating to tighter osteotomies. After 6 weeks, RTQ values for the M and T groups were the highest, but no statistical significance was detected. When considering the change in removal torque values

over time, a significant increase in torque values was observed for group R between 3 and 6 weeks ($p<0.05$).

Histologic and histomorphometric evaluation

The histological analysis presented new bone formation in close contact with the implant surfaces for all group at both observations times (Figure 2). At 3 weeks, the implants inserted in tight osteotomies (group T) were in close contact mainly with cortical bone, while the implants in the group R were surrounded by newly formed bone and loose acellular tissue. For all groups, areas of bone apposition were visible in the area within the threads and newly formed woven bone, with numerous highly stained osteocytes were present in all sections. At 6 weeks, less differences among and between groups were visible as new and mineralized bone was in contact along a substantial portion of the bone-implant interface.

The mean bone to implant contact (BIC) percentages $\pm$ SD for each group are presented in table (Table 2) and graph (figure 3a). In general, an increase in bone contact over time was noticed, although the increase was significant only for group M ($p<0.05$). Higher BIC values corresponded to the different instrumentation procedure, order from the loosest to the tightest preparation. The mean differences among the groups did not reach significant value sat 3 or 6 weeks, but when the BIC values of both time points were pooled, a significant trend of increasing bone-contact for the ordered groups was found ($p<0.05$).

Descriptive statistics for the bone area fraction occupancy (BAFO) are presented in Table 3 and Figure 3b. Again, a general increase over time was observed for BAFO values and a tendency of increased values for the groups ordered from R to T. At 3 weeks of healing the trend of higher bone filling between instrumentation

groups ordered from R to T was statistically significant ($p < 0.05$), while the difference was not significant between groups at 6 weeks ($p > 0.05$). When the BAFO results at both time points were pooled, the level of significance increased between groups ($p < 0.05$).

4. Discussion

This experiment investigated the effect of different osteotomy preparations on the osseointegration of an implant with microrough surfaces obtained with resorbable blasting media (RBM).

This RBM surface treatment induces topographical changes in combination with chemical alterations, due to the incorporation of CaP particles onto the titanium surfaces³⁰. Previous studies have demonstrated improved host-to-implant responses for implant treated with resorbable blasting media to be comparable to other moderately rough surfaces¹⁸.

Besides microgeometry, implant macrogeometry has a significant effect on the healing process through implant primary stability. For each unique combination of modified implant surface and different microgeometry, implant companies suggest a specific surgical protocol. The initial distance created between the implant surface and the bone bed is thought to induce different bone healing phenomena³¹. When the osteotomy is smaller than the implant diameter, the mechanical friction is high and the implant micromotion is reduced³². The predominant process in the bone regeneration is the remodeling of the original bone through osteoclasts activity and the substitution with new bone. During the uninterrupted healing, there is a gradual shift from the implant primary stability, the early mechanical interaction between the bone bed and the implant, and the secondary stability given by biological

integration³³. The least stable moment in this process is weeks after implant placement, when mechanical interlocking drops due to viscoelastic relaxation of the bone along with remodeling of the bone close to the implant interface²⁶.

On the other hand, when the implant is placed after preparation with full drilling sequence, the difference in diameter between the implant inner thread diameter and drilling outer diameter results a closed chamber that is filled with blood and blood clot, which interact with the whole implant surface, and bone formation proceeds for contact osteogenesis without extensive interfacial remodeling⁴.

In the present study, an increase in osseointegration measurable parameters such as BIC and BAFO from the loosest to the tightest preparation was observed. On the other hand, the removal torque testing showed significantly higher retention for implants inserted in tighter osteotomies after 3 weeks of healing. This finding is consistent with the higher bone-area fraction occupancy (BAFO%) of the press-fit groups (Medium and Tight), which had significantly more bone within the implant threads. The removal torque scores further increased at 6 weeks for all groups, indicating that the implant stability was maintained at a high level during the healing process and the shift from primary to secondary stability did not undermine the implant anchorage. However, only for the R group, the increase of RTQ scores from 3 to 6 weeks was statistically significant, indicating that new bone successfully formed around the implant in this drilling condition.

Histologic slides displayed an intimate contact between bone and the implant surfaces, at both time points. The osteoid secretion by active osteoblasts was evident in the histologic sections, and they surrounded the implants along the entire surface. Newly formed woven bone visible on the sections at 3 weeks was entirely substituted by mineralized bone in the 6-weeks sections. The high bone-contact rates indicate the

biocompatibility and osteoconductivity of the moderately roughed and chemically modified RBM surface regardless of drilling protocol. Furthermore, the potential presence of residual CaP particles from the surfaces may have induced osteogenic cell recruitment and activation contiguously to the implant.

These results confirmed that a simplified drilling protocol does not have negative indications and it achieves at least comparable or faster osseointegration than the conventional drilling procedure, with gradual expansion of the implant site^{34, 35}.

Conclusion

The results of the study confirmed the biocompatibility and successful integration of microrough and CaP-doped titanium surfaces obtained by resorbable media blasting. Moreover, it can be concluded that a simplified surgical procedure, which reduces the drill sequence and generates a press-fit situation, achieved high primary stability and led to successful bone regeneration and stable biological fixation of the implants.

Table 1: RTQ values at 3 and 6 weeks (values in cNm) for the four test groups (R, L, M and T). The p-values were obtained with Page's L trend test for ordered related samples.

		R	L	M	T
3 weeks	Mean	74.17	73.83	94.50	91.67
	Standard Deviation	22.03	21.39	16.27	16.43
	Maximum	100.00	109.00	110.00	119.00
	Minimum	45.00	50.00	64.00	69.00
	p-value	0.02			
6 weeks	Mean	101.33	93.33	107.50	113.33
	Standard Deviation	17.11	10.37	11.26	28.08
	Maximum	127.00	110.00	119.00	166.00
	Minimum	84.00	81.00	88.00	81.00
	p-value	0.52			
p-value (pooled 3- and 6-weeks results)		0.04			

Table 2: BIC values (%) at 3 and 6 weeks for the four test groups (R, L, M and T).

The p-values were obtained with Page's L trend test for ordered related samples.

		R	L	M	T
3 weeks	Mean	45.07	45.86	51.77	54.81
	Standard Deviation	17.54	12.76	14.80	14.20
	Maximum	58.51	62.82	66.59	74.44
	Minimum	10.70	30.19	24.58	37.50
	p-value	0.2			
6 weeks	Mean	55.69	57.44	66.43	65.79
	Standard Deviation	8.64	11.42	5.91	5.89
	Maximum	69.90	69.18	74.80	72.80
	Minimum	46.55	43.04	59.85	55.25
	p-value	0.08			
p-value (pooled 3- and 6-weeks results)		0.03			

Table 3: BAFO values (%) at 3 and 6 weeks for the four test groups (R, L, M and T).

The p-values were obtained with Page's L trend test for ordered related samples

		R	L	M	T
3 weeks	Mean	37.56	41.08	45.55	49.49
	Standard Deviation	13.43	13.52	10.14	15.18
	Maximum	58.60	52.60	56.70	68.17
	Minimum	19.00	21.55	31.67	25.07
	p-value	0.03			
6 weeks	Mean	44.69	41.35	57.52	60.83
	Standard Deviation	12.48	14.17	15.14	9.97
	Maximum	64.69	58.61	75.14	74.06
	Minimum	28.90	22.33	40.59	43.26
	p-value	0.3			
p-value (pooled 3- and 6-weeks results)		0.02			

Figure Legends

Figure 1

Removal torque (adjusted estimates $\pm$ 95% CI) as a function of instrumentation and healing time.

Figure 2

Histologic observations (bars: 200 μ m) of the effect of 4 different instrumentation procedures. (a) After 3 weeks. (b) After 6 weeks.

Figure 3

(a) Bone-to-implant contact (BIC, adjusted estimates $\pm$ 95% CI) as a function of instrumentation and healing time. (b) Bone area fraction occupancy (BAFO, adjusted estimates $\pm$ 95% CI) as a function of drilling technique and healing time.

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Fig. 1

Fig. 2a

Fig. 2b

Fig. 3

Fig. 4