ALI ALENEZI

**ON ENHANCEMENT OF BONE
FORMATION USING LOCAL
DRUG DELIVERY SYSTEMS**

**ON ENHANCEMENT OF BONE FORMATION
USING LOCAL DRUG DELIVERY SYSTEMS**

Malmö University
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Malmö University, 2018
Faculty of Odontology
Department of Prosthodontics
Malmö, Sweden

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To my beloved wife Dalal,
my precious kids Johanna and Basil,
and my father and mother.

This thesis is number 52 in a series of investigations on implants, hard tissues and the loco-motor apparatus originating from the Department of Biomaterials, University of Gothenburg and the Department of Prosthodontics/Material Sciences, Malmö University, Sweden.

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THESIS AT A GLANCE

Study	Aim	Illustration	Key findings
(I) Effects of local drug and chemical compound delivery on bone regeneration around dental implants in animal models: a systematic review and meta-analysis	To evaluate the methods most commonly used for local drug and chemical compound delivery to implant sites and assess their influence on osseointegration.		The use of local chemical compound delivery systems around implants could significantly improve implant osseointegration in animal models.
(II) Development of a Photon Induced Drug- Delivery Implant Coating	We have developed a drug delivery system using a thin GNR- containing PNIPAAm coating. Using NIR as an external stimulus, drug release can be initiated and controlled		Proof-of-concept of the drug-delivery system was demonstrated by releasing the antibiotic vancomycin to eradicate <i>Staphylococcus epidermidis</i> bacteria in culture
(III) Controlled release of clarithromycin from PLGA microspheres enhances bone regeneration in rabbit calvaria defects	To confirm the sustained release of clarithromycin from PLGA microspheres within beta-TCP in vitro and to examine the efficacy of them as the bone substitute materials in rabbit a calvaria defect model		PLGA microspheres loaded with clarithromycin was suggested as a novel drug delivery system for sustained drug release to enhance bone regeneration in the rabbit calvaria defect model used in the current study
(IV) Osseointegration effects of local release of strontium ranelate from implant surfaces in rats	To evaluate whether the release of strontium ranelate (Sr- ranelate) from implants coated with mesoporous titania could improve bone formation around implants in animals model.		The mesoporous Ti film allowed sustained release profile of Sr- ranelate. This study could not confirm the positive effects of Sr-ranelate on implant incorporation in bone made by other authors.

ABSTRACT

Introduction: Despite that many reports have confirmed the long-term clinical success rates associated with implant treatment, implant failure to achieve and maintain osseointegration still occurs in many cases. Local and sustained drug release at the bone-implant interface is one of the strategies that have been suggested to improve the osseointegration. The local drug release could help avoiding the risks usually associated with systemic administration, such as high drug dose or the loss of drug bioavailability.

Aims: To map out the most commonly used chemical compounds and drug delivery systems used in animal experiments for implant research (Study I). Furthermore, to develop a new surface coating designed for medical devices and implants, and to examine the drug release mechanism from the coating using near infrared light (NIR) as an external stimulus (Study II). In addition, we examined the release of clarithromycin from PLGA microspheres within beta-tricalcium phosphate (β -TCP), and evaluated their osteogenic effect in a calvaria defect model *in vivo* (Study III). In Study IV, we evaluated the local release of strontium ranelate (Sr-ranelate) from implant surface coated with mesoporous titania films, and investigated if the local release of Sr-ranelate could improve bone formation around implants in an animal model.

Materials and Methods: The articles included in the present thesis consist of four different studies. For Study I, an electronic search

was done in three databases (PubMed, Scopus, Embase) to map out the most commonly used methods for local drug and chemical compound delivery to implant sites, and to assess their influence on bone response. Meta-analyses were performed for the outcome of bone-to-implant contact (BIC). In Study II, PNIPAAm-AAm polymers were synthesized at different compositions. The polymers were then incorporated with gold nanorods (GNRs) since these rods at predetermined aspect ratio can absorb NIR light to generate heat within the polymeric layer to initiate a drug release. The volume-phase transition behavior for the polymers was analyzed using differential scanning calorimetry (DSC). The GNRs-incorporated PNIPAAm was characterized using scanning electron microscopy (SEM) and quartz crystal microbalance with dissipation monitoring (QCM-D). The release behavior using phenol as drug model was investigated upon NIR irradiation using UV/VIS spectroscopy. In addition, the antibacterial behavior of polymer layers loaded with vancomycin was examined against *Staphylococcus epidermidis*. In Study III, four bone defects (5 mm of diameter) were created in the calvaria of New Zealand White rabbits (n = 21, n = 7/time point). The defects were randomly designated to four groups. Group 1: no augmentation (sham), Group 2: β -TCP, Group 3: β -TCP with 0.12 mg clarithromycin, and Group 4: β -TCP with 6.12 mg PLGA microspheres loaded with 0.12 mg clarithromycin. After 2, 4, and 12 weeks of healing, bone regeneration was evaluated using micro-computed tomography (μ CT) and histology. In Study IV, mini-screw titanium implants were coated with mesoporous TiO_2 films using Pluronic (P123) with or without poly propylene glycol (PPG) to create films with two different pore sizes. The implants were then incorporated with Sr-ranelate. SEM evaluation was performed to visualize the mesoporous TiO_2 films and determine the pore size. The absorption and release kinetics of Sr-ranelate from mesoporous TiO_2 films were evaluated by QCM-D. For the *in vivo* experiment, mini-screw titanium implants with or without Sr-ranelate were inserted in rats' tibia bone to evaluate bone formation after 2 and 6 weeks.

Results: In the systematic review (Study I), sixty-one studies met the inclusion criteria. Calcium phosphate (CaP), bisphosphonates (BPs), and bone morphogenetic proteins (BMPs) were the most commonly used chemical compounds. There were two main methods for local drug delivery at the bone-implant interface: (1) directly from an implant surface by coating or immobilizing techniques, and (2) the local application of drugs to the implant site, using carriers. There was a statistically significant increase in BIC for both local drug delivery methods ($p = .02$ and $p < .0001$, respectively) when compared to controls. There was a statistically significant increase in BIC when CaP ($p = .0001$) and BMPs ($p = .02$) were either coated into implants or delivered to the implant site, in comparison to when drugs were not used. The difference was not significant for the use of BPs ($p = .15$). In Study II, the DSC analyses showed that PNIPAAm-AAm containing 10% acrylamide had an appropriate phase transition temperature of 42°C. SEM images showed that the surface coating consisted of a 200 nm thick uniform polymer layer. The QCM-D analysis coupled with *in situ* NIR irradiation demonstrated a dramatic shift in frequency that was attributed to mass being released from the surface upon irradiation. This mass release correlated well with the drug release profile as determined using UV/VIS spectroscopy with phenol as a model drug.

For Study III, clarithromycin release from PLGA microspheres revealed sustained release for around 4 weeks with 50% release during the first week. Histologically, new bone formation was evident at 2 and 4 weeks of healing in all groups and bone formation increased as a function of healing time. At 12 weeks, Group 4 (β -TCP with PLGA microspheres loaded with clarithromycin) showed significantly higher amount of newly formed bone compared to Group 1 (sham). The μ CT showed that Group 4 expressed significantly higher bone formation compared to Group 1 at all time points.

In Study IV, the SEM images showed TiO₂ films with porous structures covering the entire surface with pore sizes determined to be 6 nm for P123 and 7.2 nm for P123-PPG. The QCM-D analysis revealed an absorption of 3300 ng/cm² of Sr-ranelate on the 7.2 nm

TiO₂ films, which was about 3 times more than the observed amount on the 6 nm TiO₂ films (1200 ng/cm²).

The histomorphometric analyses revealed higher percentages of bone implant contact (BIC) and bone area (BA) for implants with Sr-ranelate compared to implants in the control group after 2 and 6 weeks of healing. However, these differences were found not to be significant (BIC with a *p*-value of 0.43 after 2 weeks and 0.172 after 6 weeks), (BA with a *p*-value of 0.503 after 2 weeks, and 0.088 after 6 weeks). The mean BIC and BA values within the same group showed significant increase among all groups after comparing 2 and 6 weeks.

Conclusions: Most studies assessing local drug/chemical compound release systems in implants evaluated the influence of the use of BPs, CaP, and BMPs on bone healing. The use of local chemical compound delivery systems around implants could significantly improve implant osseointegration in animal models (Study I). In addition, on demand-release of the antibiotic agent vancomycin from the coating induced by NIR light resulted in a clear inhibition zone around a coated substrate in a bacteria culture test, thereby providing proof of concept of the developed drug delivery system (Study II). The *in vivo* findings showed that β -TCP with clarithromycin-loaded microspheres can enhance bone formation in bone defects (Study III). Meanwhile, the *in vivo* findings on Sr-ranelate study (Study IV) could not confirm the positive effects of Sr-ranelate on implant incorporation in bone made by other authors.

LIST OF PAPERS

This dissertation is based on the following four 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. Effects of local drug and chemical compound delivery on bone regeneration around dental implants in animal models: a systematic review and meta-analysis.
Alenezi A, Chrcanovic B, Wennerberg A.
The International Journal of Oral & Maxillofacial Implants. 2018 Jan/Feb; 33(1): e1-e18.
- II. Development of a Photon Induced Drug-Delivery Implant Coating.
Alenezi A, Hulander M, Atefyekta S, Andersson M.
(Submitted).
- III. Controlled release of clarithromycin from PLGA microspheres enhances bone regeneration in rabbit calvaria defects.
Alenezi A, Naito Y, Terukina T, Prananingrum W, Jinno Y, Tagami T, Ozeki T, Galli S, Jimbo R.
Journal of Biomedical Materials Research. Part B, Applied Biomaterials, 2018. 106(1): p. 201-208.

- IV. Osseointegration effects of local release of strontium ranelate from implant surfaces in rats.
Alenezi A, Galli S, Atefyekta S, Andersson M, Wennerberg A.
(Submitted).

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Contribution by the respondent

The respondent performed most of the work from planning, experimental work (with exception of the surgical procedures with animals, clarithromycin release test, and the μ CT analyses) and he performed the analysis of the data. The respondent was also the main contributor to writing the manuscripts.

ABBREVIATIONS AND ACRONYMS

c.p. Ti	Commercially pure titanium
PEEK	Polyether ether ketone
BMPs	Bone morphogenetic proteins
GDFs	Growth and differentiation factors
HA	Hydroxyapatite
AFM	Atomic force microscopy
SEM	Scanning electron microscopy
Lenti-BMP-2	Lentiviral vector encoding BMP-2
MT	Mesoporous titania
PLGA	Poly-(lactic-co-glycolic acid)
μm	Micrometer
PNIPAAm	Poly (N-isopropylacrylamide)
NIPAAm	N-isopropylacrylamide
PNIPAAm-AAm	Poly(N-isopropylacrylamide) -co- acrylamide
LCST	Lower critical solution temperature
PNIPAAm-co-AAc	Poly(N-isopropylacrylamide-co-acrylic acid)
WSC	Water-soluble chitosan
MSCs	Mesenchymal stem cells
GNRs	Gold nanorods
NIR	Near infrared
nm	Nanometer

μM	Micromolar
mW	Milliwatts
MRI	Magnetic resonance imaging
EPR	Electron Paramagnetic Resonance
NMR	Nuclear magnetic resonance
UV/VIS	Ultraviolet–visible
QCM-D	Quartz crystal microbalance with dissipation monitoring
Δf	Change in frequency
Δm	Change in mass
Hz	Hertz
BIC	Bone-to-Implant Contact
SD	Standard deviation
BA	Bone area
new-BA	New bone area
AIBN	<i>N,N'</i> -Azobisisobutyronitrile
DCM	Dichloromethane
milli-Q	Millipore
PVA	Polyvinyl alcohol
O / W	Oil / water
rpm	Rotation per minute
P123	Pluronic® P123, triblock copolymer EO20PO70EO20
PPG	Poly propylene glycol
CTAB	Cetyl trimethylammonium bromide
Mn	Molecular weight
nM	Nanomolar
mM	Millimolar
DSC	Differential scanning calorimetry
kV	Kilovolt
PEG	Poly(ethylene glycol)
W/cm²	Watt/square centimeter
PBS	Phosphate-buffered saline
D₂O	Deuterium oxide
ng/ cm²	Nanogram/ square centimeter

$\mu\text{g}/\text{cm}^2$	Microgram/ square centimeter
Sr-ranelate	Strontium ranelate
μCT	Micro-computed tomography
μm^3	Cubic micrometer
ROI	Region of interest
NB	Formed new bone
CaP	Calcium-phosphate
BP	Bisphosphonate
MD	Mean difference
CI	Confidence interval
LED	Light-emitting diode
β-TCP	β -tricalcium phosphate
rhBMP-2	Recombinant human bone morphogenetic protein-2
Sr	Strontium
MSPs	Microspheres

INTRODUCTION

Background

Many studies have shown that the loss of teeth can affect the life quality ¹. This effect is not only concerning the loss of function, but has also negative psychological impact since it could affect the smile esthetic at the same time. There has been greater demand from patients for treatment simultaneously aimed at restoring teeth function and appearance, and effective treatment strategies should consider the economic factor related to the patients.

Previous treatment strategies were based mainly on using a tooth-supported fixed bridge or a removable prosthesis to replace missing teeth ². Although these treatment strategies revealed good outcomes and high patients satisfaction ³, some complications are sometimes observed. The tooth-supported fixed bridge involves multiple dental visits and preparation steps that can be harmful to the neighboring teeth. For example, some of the clinical complications include caries and the need for endodontic treatment⁴. Meanwhile, the removable prosthesis can only provide limited improvement in function compared to natural teeth ⁵. In addition, a removable prosthesis can be associated with some complications such as loss of retention and ulcerations that cause patient dissatisfaction⁶. Most Patients today have a high expectation for esthetics outcome and not simply improved function ⁷.

Over the last decades, implant materials have become a good alternative for replacing missing teeth and supporting dental prostheses ⁸. Implant restoration became popular because some of

the limitations seen with conventional treatment disappear and long-term successful treatment options are provided ⁹.

The endosseous implants can be used as foundation devices for oral prostheses in several clinical situations that vary from single crown restorations to full arch rehabilitations. Thus, implant restorations are believed to offer aesthetic and psychological benefits to the patient ^{10, 11}. Implants can resemble the natural tooth appearance more than traditional fixed prostheses while avoiding the need for removing of a sound tooth structure when preparing the adjacent teeth for the fixed prosthesis. Some reports have shown that dental restorations supported by implants are associated with increased patients satisfaction compared to conventional treatment ¹².

Furthermore, interest in implant restoration from both patients and clinicians corresponds with the high success rate, as has been reported in numerous studies. In a systematic review by Berglundh et al.,¹³ a 5-year clinical follow up revealed more than 95% implant survival. The high survival rate was also observed in another recent study (94,6%) after a longer evaluation of 10 years ¹⁴. Previously, there was a tendency to avoid placing a single implant in an area with less cortical bone to support the implant such as the posterior maxilla area because of low bone quality. Instead, single implant therapy was mainly used for anterior sites of the upper and lower arch.

The advances in implant science and the excellent clinical success in addition to the development of new operative procedures, such as sinus grafts to improve bone volume, have prompted the use of implants in broader and more challenging clinical situations ¹⁵. Albrektsson named six factors that have major influence on successful implant integration in bone, including the used surgical technique, type of material, and the healing time before implant loading ¹⁵.

Numerous implant designs have been used over time such as cylindrical and tapered implants. The cylindrical represent the only devices that have survived the scrutiny of time. The implant morphology is manufactured with threads to increase the implant area and the initial contact with bone ^{16, 17}. Maximizing the implant

bone contact may help obtain early stability but clinical correlations are uncertain. The threaded implants have been developed with different thread dimensions to allegedly improve integration and marginal bone healing ¹⁸. Optimal implant design may help minimizing the incidence of implant loosening or even fracture ¹⁹.

The standard protocol in implant therapy suggests placing an implant out of function until completing a healing period of several months. Loading of the endosseous implants has been suggested first when the bone formation around the implant is expected to be completed. Thus, a healing time around 3–6 months after the implant surgery is the standard protocol before implants are placed in function. In the case of tooth extraction, the bone healing process is estimated to require up to several months ²⁰. The major reason behind the extended healing time is to ensure the presence of satisfactory bone density before implant surgery since this factor plays a main role in successful implant treatment.

Having said this, immediate implant loading has received much interest too since it reduce chair time for patient and shortened the time before the implant can be in function ²¹. The discomfort and esthetic problems generated from having missing teeth can be minimized with this short treatment strategy. The prerequisite factors for successful implant treatment that are used in traditional protocols can be applied with immediate implant loading. However, the primary stability can be defined as the most important factor also with immediate loading ^{22,23}. A clinical study was conducted by Chiapasco et al.²⁴ in which 194 patients were followed for 2-13 years. The follow up results revealed that when osseointegration occurred, similar successful results were obtained for immediately loaded implants and implants with delayed loading. Meanwhile, a histological evaluation showed high bone-implant contact measures for implants loaded immediately after surgery.

For delayed as well as immediate loading, implant treatment needs to ensure proper outcomes for patients with the goal of restoring function and aesthetics as much as possible.

Titanium as a biomaterial

Commercially pure (c.p.) titanium is the most commonly used material for oral implants and titanium alloy is commonly used for hip arthroplasties. The interest in titanium was initiated by the early experiments by Brånemark et al. in the 1960s when they noticed unexpected difficulties to remove the implants ^{25, 26}. Since then, titanium implants have shown excellent clinical function after decades of follow up ²⁷. The titanium implant has demonstrated minimal inflammatory response and excellent biocompatibility ²⁸. The biocompatible properties are attributed to the thin oxide layer that is formed immediately on a titanium surface when exposed to oxygen in the air ²⁹. The oxide layer was reported to be a few nanometers in thickness and believed to enhance the material corrosion resistance ³⁰. The chemical composition of c.p. titanium consists of around 99.7% titanium while the remaining 0.3% is a mix of several elements such as iron, oxygen and carbon. There are four grades available for the c.p. titanium classified based on the amount of the elements included. The early experiments by Brånemark introduced the concept of osseointegration and were based on using c.p. titanium grade 1. However, most available oral implants today are manufactured using c.p. titanium grade 4 since this type of titanium contains double as much iron which makes the implant significantly stronger.

The mechanical properties of titanium materials are highly dependent on the chemical composition. For instance, titanium alloys have, according to some investigators, similar biocompatibility but stronger mechanical properties compared to commercially pure titanium ³¹. Titanium grade 5 (Ti-6Al-4V) is the most commonly used titanium alloy for different biomedical applications in orthopedics.

Early clinical studies on titanium implant reported hypersensitivity and allergic reactions with some patients have been suspected, although no reliable tests to prove this condition ³²⁻³⁴. Meanwhile, Franchi et al.³⁵ reported that friction between the implant surface and bone cavity during implant placement can lead to that Ti particles detach from the surface. If allergy to titanium exists after all, other materials have been suggested as alternative

such as zirconia, hydroxyapatite, and polyether ether ketone (PEEK)
36-38.

Bone tissue

Bone is a cellular and highly mineralized rigid connective tissue, which is subjected to continuous remodeling throughout life. In the oral cavity, different sites have different types of bone based on the ratio of cortical to trabecular bone. In this macroarchitecture, cortical and trabecular bone have the same bone composition, and they only differ in the amount of bone mass per unit volume. The porosity within the cortical and trabecular bones are approximately 10% and 50-90%, respectively. Thus, cortical bone offers greater mechanical and protective strength compared to trabecular bone. Consequently, for a long period, the areas with more cortical bone were believed to provide a better prognosis with implant treatment. In addition, a clinical classification was made to divide the bone tissue in the oral cavity based on the differences in the cortical bone and trabecular bone ratios. For instance, sites with mainly cortical bone, such as in the anterior mandible, were classified as quality 1 bone, while sites with nearly all trabecular bone, such as in the posterior maxilla, were classified as quality 4 bone ³⁹.

The bone tissue contains a mixture of organic and inorganic components, and around 10% of bone weight is composed of water. The organic components include numerous proteins (mainly collagen fibers type 1) that correspond to around 25% of bone weight. The remaining 65% comprises inorganic components that are basically the apatite crystals that contain calcium and phosphorous. Bone is considered a major reservoir for inorganic ions, and bone helps regulate the mineral homeostasis in the body. In addition, blood cells are synthesized in the bone marrow.

Osseointegration

During the 1960s, Brånemark introduced the concept of osseointegration after having placed endosseous implants in some patients and observed strong integration of the implant with bone. From a clinical perspective, osseointegration is known by the firm and stable integration of the implant with the surrounding area.

This biological outcome is desired from bone tissue healing around titanium and is considered the main sign of a successful treatment. Factors such as the implant material, patient behavior, and implant site have major roles in satisfactory implant osseointegration^{13,40}.

Numerous definitions have been used to describe osseointegration from different perspectives. From a histological perspective, one of the first definitions was given by Albrektsson et al. in 1981 who described osseointegration as the direct contact between implant and bone, when observed with an optical microscope¹⁵. Furthermore, Zarb and Albrektsson⁴¹ defined osseointegration as the following: "A process whereby clinically asymptomatic rigid fixation of alloplastic material is achieved and maintained in bone during functional loading".

Osseointegration of titanium implants was believed, for a long period, to be a result of the high biocompatibility of titanium material. However, some recent studies suggest that osseointegration is possibly a result of foreign body response in which the bone formation around titanium is actually a defense mechanism to encapsulate the foreign material⁴², which is behind a new definition of osseointegration revealed by Albrektsson et al.⁴³ who described osseointegration as: "a foreign body reaction where interfacial bone is formed as a defense reaction to shield off the implant from the tissues".

The osseointegrated implant is characterized by strong fixation with bone that is expected to work well under normal loading for an extended amount of time⁴⁴. This fixation is believed to be the result of a strong interaction between the bone and the titanium oxide layer. In general, after some time in situ it may prove difficult to separate the implant from the surrounding bone without causing fracture. Thus, these findings promote the use of implants as an abutment to support dental prostheses. On this basis, awareness of the mechanisms that involve bone formation after implant surgery are important to understand, particularly for cases with delay or shortage in bone formation. Numerous *in vivo* and *in vitro* studies have discussed the healing process of implants. A successful implant treatment was seen to be highly dependent on two types of stability, namely the primary and secondary stability⁴⁵.

Primary stability is characterized by mechanical fixation of the implant with the surrounding bone shortly after insertion; the absence of implant movement is essential for bone healing process⁴⁶. The principle of primary stability is similar to the one used for treating fractured bone, in which stabilizing the fractured parts in place is required to promote fracture healing. The presence of movement could cause stresses that negatively affect bone remodeling and causes fibrous tissue formation at the implant interface ⁴⁷. Thus, some older strategies suggested the use of a stabilization device to enhance the primary stability of implants such as applying a splint device to prevent micromotion of the implant after surgery ⁴⁸. In contrast, the secondary stability, also known as biological stability, involves a sequence of bone regeneration and remodeling and biomechanical interlocking ⁴⁹.

Healing process

The healing of an osseointegrated implant has been described by Schroeder et al.⁵⁰ as “functional ankylosis” of the implant-bone interface. This ankylotic interface is formed through a series of healing phases starting from the moment the bone is drilled for implant placement. The period of bone healing around the implant can take up to many months including a long dynamic bone remodeling stage. Understanding the process of peri-implant bone healing is highly important for clinicians to accurately evaluate the causes of early implant failure.

Healing of the wound around an endosseous implant is believed to be somehow similar to the healing observed after bone fracture, which involves a series of coordinated and organized healing mechanisms ⁵¹. The difference in the case of implant healing is that the host response is modified by the presence of a foreign body, i.e., the implant. In addition, material biocompatibility and surface characteristics can significantly influence the healing steps.

Numerous studies have discussed the healing process around implants and the effects of different surface topographical and chemical properties on the tissue response ^{52, 53}. However, most information regarding the healing process around implants has been obtained using *in vivo* and *in vitro* studies ⁵⁴. Some of the

mechanisms involved in the bone healing around the implants surface are yet not fully understood.

In some animal studies, histological observations of different types of cells around titanium implants during bone formation has been seen ⁵⁵. Growth factors and cytokines are also involved during different phases of bone healing ^{56, 57}. The cellular and biological events occurring at the bone-implant interface and regulated by different molecules like growth factors released by blood cells ^{58, 59}. In addition, bone healing around an implant can demonstrate the same phases as fracture bone healing: the haemostasis phase, the inflammatory phase, the proliferative phase, and the bone remodeling phase (Figure 1).

Figure 1. Mechanisms of bone healing and regeneration around implant.

Haemostasis: The process of bone healing is believed to begin during the bone drilling to prepare the implant site, which causes rupture of the surrounding blood vessels resulting in hemorrhage. The bleeding helps establish a series of biological processes to initiate tissue formation. The interaction between blood cells and implant surface activate numerous platelets shortly after contact with blood

⁶⁰. In addition, some studies have suggested that implant topography can influence the degree of platelet activation ⁶¹. For instance, implants with more microtopographical features will reveal more platelet activation compared to smoother surfaces. The activated platelets help the prothrombin to produce thrombin. Then, thrombin generation will facilitate fibrinogen conversion to fibrin in a process known as secondary hemostasis ⁶². Platelet activation also causes the platelets to aggregate and form a clot. This is an important process since the formation of stable blood clot is needed for recruiting osteogenic cells ⁶³. In addition, the implant surface design can influence fibrin retention, and a fibrin clot works as a transition point between hemostasis and the inflammatory phase.

Inflammatory phase: The inflammatory phase occurs simultaneously with platelet activation ⁶⁴. Blood cells and inflammatory cells migrate from the surrounding vessels to the tissue surrounding the implant. In addition, cytokines and other growth and differentiation factors are released by blood cells at the bone implant interface ⁵⁹. Other cells, such as neutrophils followed by monocytes, will migrate into the peri-implant space within 24 hours. The platelets release cytokines that will activate the leukocytes from the surrounding capillaries⁶⁵. Growth factors including bone morphogenetic proteins (BMPs) and growth and differentiation factors (GDFs) are expressed within 24 hours ⁶⁶. These factors have been reported to promote bone formation, and vascularization may be very crucial for tissue differentiation and for metabolically active osteogenic cells⁶⁷.

Proliferation phase: Mesenchymal cells are locally recruited from bone marrow and pericytes and then migrate through the fibrin clot and other matrix proteins toward the implant surface ⁶⁸. Numerous factors released by platelets and leukocytes will cause mesenchymal cells to differentiate into osteoprogenitor cells ^{69, 70}. For example, osteoblasts and mesenchymal cells synthesize *de novo* bone and deposit bone-related proteins on the implant surface. These cells also form a non-collagenous matrix layer on the implant surface that is useful in regulating cell adhesion and mineral binding ⁷¹.

Afterwards, the bone cells stop migrating and they start secreting mineralized tissue on the surface of the implant (contact osteogenesis) and on the surface of the old bone (distance osteogenesis)⁷². Then, fully differentiated osteoblasts form a collagen containing layer. The osteoblast is sometimes enveloped during the mineralization process and becomes an osteocyte within a bone lacuna³⁵. The mineralization process into the collagen layer will produce woven bone in two opposite directions.

Remodeling phase: The bone contacting the implant surface is continuously being transformed throughout the lifetime. Bone remodeling involves cycles of bone resorption and bone formation that work as a self-repair mechanism. It has been suggested that bone remodeling includes four different processes: osteoclasts activation, bone resorption, osteoblasts activation, and mineralization of new bony tissue ⁷³. Osteoblasts and osteoclasts have couple functionality and need one another for proper bone remodeling ⁷⁴. The same cells are immunologically active too ⁷⁵.

Excessive loading of the implant could impair early bone healing by causing damage to the surrounding fibrin network and vasculature⁷⁶. Eventually this damage could lead to implant mobility and marginal bone loss and probably implant failure. Today, an implant with direct contact with bone with no progressive relative mobility is considered as an osseointegrated implant.

Why the need for improvement

Many reports have confirmed the long-term clinical success rates associated with implant treatment. Subsequently, the success rates have increased the popularity of implant treatment and encouraged the use of the implant treatment to help solve more challenging clinical situations ⁷⁷. However, failure to achieve and maintain osseointegration still occurs due to several reasons ⁷⁸. Patients with compromised medical conditions such as diabetes mellitus have lower implant survival rates, which could be attributed to the impaired healing system ⁷⁹. Post-menopausal women can suffer from osteoporosis characterized by enhanced bone resorption activity, leading to low implant stability and failure ^{80, 81}. In addition,

situations with poor bone quality could require specific implant design and particular techniques to enhance implant treatment.

Meanwhile, some complications such as biofilm infections are sometimes seen around implants. Implant-associated infections are difficult to treat, and they can be associated with inflammatory reaction that may complicate bone healing around implants ⁸². Insufficient treatment for such cases may lead to implant failure in the long-term ⁸³⁻⁸⁵. Thus, further research is needed to provide proper treatment strategies to minimize the complications associated with infections and to improve the long-term fixation of implants ⁸⁶.

Strategies to enhance bone response

A common strategy to improve implant healing is by modifying surface topography and chemical properties to potentially improve host tissue responses. For example, implant topography and surface chemistry can influence conformation and adsorption of proteins ^{71, 87}, which may stimulate cellular response toward new bone formation ⁸⁸.

In the past, a machined implant surface was largely used in implant dentistry. However, most commercially available implants currently show moderately rough surfaces. Different techniques can be used to obtain moderately rough surfaces such as grit blasting, acid etching, anodization, and combinations of these techniques ⁸⁹⁻⁹². Some of the methods numerously used for surface modifications, such as hydroxyapatite (HA) coating, can produce chemical and topographical modifications at the same time ⁹³. These modifications further revealed different surface energies. Moderate surface roughness was suggested to enlarge surface area and enhancing implant primary stability⁹⁴. This surface enlargement was believed to improve bone formation compared to machined surfaces ⁹⁵. In addition, surface roughness may stimulate cellular responses by enhancing osteoblast attachments and differentiation ^{96, 97}.

Blasting particles combined with acid etching is a frequently used technique to modify the surface topography ^{98, 99}. With the blasting technique, the surface roughness can be changed by

controlling the velocity of the particles, particle size, and particle properties¹⁰⁰. The added acid etching can help clean off the remnants of the particle, and alter the surface chemistry and topography^{101, 102}. Some *in vivo* studies reported increased surface area using this type of surface modification that resulted in improved bone regeneration^{98, 103, 104}. Moreover, some chemically modified surfaces were hypothesized to deliver a biochemical bonding between the implant and the surface^{105, 106}, although a precise evidence for this bonding is lacking.

Anodic oxidation is another commonly recognized method to chemically and topographically modify the surface. This results in an increased oxide layer thickness with a highly porous surface. Anodizing techniques were employed in some studies for incorporating different elements such as magnesium and phosphates from the electrolyte¹⁰⁶⁻¹⁰⁸. Calcium-incorporated oxidized implants demonstrated significant improvement in the rate and quality of osseointegration¹⁰⁵.

Recently, surface modifications at the nanometer resolution level has received increased interest as an implant surface with nano features affects the early bone healing process^{109, 110}. In the literature, a nanostructured material is defined as any materials showing structures with dimensions in the range of 1 to 100nm. Increasing the surface topography at a nanometer level may simultaneously alter surface area, surface energy, and wettability of the surface, which may lead to increased sites for protein adsorption and improve cellular interactions¹¹¹. Subsequently, cell and growth factor adhesion to the surface is enhanced. Increased surface nano-topography also modulates the process of cells proliferation and differentiation, facilitating bone healing around the implants¹¹². Webster et al.¹¹³ reported improved osteoblast adhesion associated with the increased surface nanoroughness.

Various modification methods can be used to obtain surface features on nanometer level, such as grit blasting, acid etching, and anodization¹¹⁴. Nanoscale TiO₂ nanotubes have been formed on an anodized Ti surface, and they significantly increased osteoblast adhesion compared to the control implant¹¹⁴. Furthermore, commercially pure Ti implants were coated with nanostructured

TiO₂ films by chemical vapor deposition and were inserted into rabbit femoral bone ¹¹⁵. These implants demonstrated increased osseointegration rate and bone mineralization.

Several studies have shown that most of the methods used induce surface modifications at micrometer and nanometer levels at the same time (Figure 2) ^{110, 116}. However, we lack clinical evidence of any superiority of nanometer alteration of the surface.

Figure 2. An implant surface that was anodic oxidized with calcium and phosphate incorporation that allowed surface modification at micro level (AFM image at the top) and nano level (SEM image at the bottom). (Alenezi et al. 2013).

One of the recent strategies to improve implant treatment is by the delivery of drug agents during implantation. The conventional treatments are by using the oral and other systemic administration ¹¹⁷, using tablets, by injection, or inhalation routes. These typical approaches involve general distribution of the drug to the entire body ¹¹⁸. However, conventional methods of drug administration

may present problems and limitations such as unwanted side effects, toxicity, and poor biodistribution ¹¹⁹.

Local drug release around implants

As an alternative, local drug release directly to the site of implantation is a promising method ^{120, 121}. This method can be beneficial to patients by providing effective doses in small amounts and reducing the risks associated with systemic administration¹²². Furthermore, several strategies have been examined to enhance implant anchorage by local release of therapeutics such as antibiotics and bone stimulating agents. These methods were based mainly on coating or incorporating drugs agents and chemical compounds on the surface of the implant for direct release of these agents into the bone-implant interface.

Drug agents and other chemical compounds can be incorporated into the implant surface using different techniques, such as plasma spray, sol-gel dip-coating, and biomimetic methods ¹²³⁻¹³⁰. Osteogenic drugs are agents that have been extensively investigated for local applications on implant surfaces. Bisphosphonate is a bone-antiresorptive drug and is believed to be very useful in improving implant osseointegration. In particular, *in vivo* study have shown that bisphosphate-loaded implant surfaces revealed increased bone mass around the implant ¹³¹, and clinical study have indicated some promises ¹³².

Many studies have investigated growth factors with dental implant to improve the healing and facilitate bone growth. Among these growth factors, bone morphogenetic proteins (BMPs) are known to support new orthotopic and ectopic bone induction ¹³³. Among them, BMP-2 is the most commonly investigated since it was reported to have superior bone induction powers ^{134, 135}. Electrostatically immobilized BMP-2 on a heparinized Ti surface was performed and revealed significantly greater bone density and bone to implant contact compared to controls ¹³⁶. However, some earlier ¹³⁷ and recent ¹³⁸ data indicate that the promise from external administration of BMPs was never fulfilled. In another animal experiment using rabbits, BMP-2 was added on implants coated with hydroxyapatite. However, six weeks after implant

installation, less peri-implant bone formation was observed for implants with BMP-2 ¹³⁹.

Some studies have focused on preparing implants with special nanofeatures such as porous structures to be utilized as platforms for drug delivery ^{140, 141}. These porous structures can be produced by electrochemical anodization of different materials such as aluminum and titanium and their alloys ¹⁴²⁻¹⁴⁴. These porous materials can be fabricated to demonstrate high surface area and loading capabilities with controllable pore and nanotube structures ¹⁴⁵. Furthermore, Malmström et al.¹⁴⁶ investigated *in vivo* the effect of open micropores in a hydroxyapatite (HA) scaffold on bone healing. Their study indicated that the open scaffold microporosity had a significant effect on bone ingrowth and bone contact inside the micropores HA group.

Surface nano-topographies such as TiO₂ nanotube coatings have been widely studied and reported to stimulate bone formation ^{114, 147-149}. These coatings can be formed by anodization and have been shown to significantly alter cell behaviors such as; cell adhesion, differentiation, and migration ^{149, 150}. In addition, TiO₂ nanotubes exhibited positive apatite-forming abilities, making them attractive substrates for bone regeneration ¹⁵¹. Lenti-BMP-loaded TiO₂ nanotube coatings were examined in real-time quantitative polymerase chain reaction and have demonstrated increased osteogenic differentiation of bone marrow stromal cells ¹⁵². Meanwhile, porous hydroxyapatite coatings were loaded with gentamicin to investigate the antimicrobial effect in an infection model in rabbits ¹⁵³. Consequently, gentamicin-coatings have shown significantly improved infection prophylaxis.

Mesoporous TiO₂ coatings consisting of films that are a few hundred nanometers thick with homogeneous nanofeatures have attracted attention as a modification strategy in implant research¹⁵⁴. One of the interesting features of mesoporous TiO₂ coatings is their highly porous surface, allowing loading and releasing of several bioactive substances^{155, 156}. Two osteoporosis drugs (Alendronate and Raloxifene) were loaded into mesoporous TiO₂ implant coatings and were evaluated *in vivo* in rat tibia¹⁵⁷. The drug-loaded implants showed significantly improved bone fixation. In addition, Galli et

al.¹⁵⁸ examined *in vivo* titanium implants coated with thin mesoporous TiO₂ films loaded with magnesium. The local release of magnesium was associated with greater bone formation in rabbit bone after three weeks of evaluation.

Polymers for local drug release

To control the drug release profile, some strategies have been examined using different carriers that can be incorporated in implants or bone substitutes. These carriers were suggested to work as local drug-delivery systems to overcome some limitations that often depend on implant coating or surface incorporation of drug agents. One such limitation is the often-occurring undesired burst in the drug release, shortening the period of drug availability and an initial too high concentration.

For materials used in local drug delivery applications, it is important to demonstrate excellent biocompatibility and low antigenic properties ¹⁵⁹. These materials should further display an ability to transport the therapeutic agent to the target tissue and allow drug release at a controlled rate and duration^{160, 161}.

Polymeric materials have attracted attention as carriers for drugs with implants and bone substitutes. The polymeric networks can imbibe large amounts of drugs and are useful for sustaining and controlling the release of a wide range of drugs. Polymers can generally be classified into biodegradable or non-biodegradable materials. The drug release mechanism from biodegradable polymer materials often occur by diffusion or erosion mechanisms. The diffusion process is influenced by some factors such as the molecular size and charge of the drugs and are characterized by drug diffusion through water-filled pores and through the swollen polymer¹⁶²⁻¹⁶⁴. The erosion process involves two main mechanisms: surface erosion and bulk erosion. Both erosion mechanisms are seen in most biodegradable polymers (Figure 3) ¹⁶⁵. The polymer chemical structure can play a major role in the extending of surface or bulk erosion. Drug release through bulk erosion occurs when water diffuses rapidly into the polymer leading to hydrolysis of polymer bonds and mass loss in the polymer bulk material ¹⁶⁶.

When the erosion rate surpasses the rate of water permeation into the bulk of the polymer, surface erosion can be observed¹⁶⁵.

Figure 3. Illustration of the drug release mechanism from biodegradable polymers through (a) surface erosion and (b) bulk erosion. Bulk erosion occurs when water diffuses rapidly into the polymer leading to hydrolysis of polymer bonds and loss of mass throughout the polymer bulk material. Surface erosion occurs when the erosion rate surpasses the rate of water permeation into the bulk of the polymer (Modified from Dinarvand et al.2011).

Some biodegradable polymers such as chitosan are used frequently with oral implants to support bone response ^{167, 168}. Chitosan is a polycationic polysaccharide that has shown good biocompatibility and antibacterial properties ¹⁶⁹⁻¹⁷¹. For example, Gulati et al.¹⁶⁷ examined chitosan and poly(lactic-co-glycolic acid) coatings on titanium surfaces loaded with an anti-inflammatory drug to improve drug release. The polymer coating improved the drug release characteristics by reducing burst release and significantly extending the overall release. Moreover, the polymer-modified implants showed excellent osteoblast adhesion and cell proliferation. In another study, chitosan-collagen composites were

wrapped onto titanium implants to evaluate their osteoinductive effect¹⁷². The chitosan-collagen composites showed ectopic new bone formation with the absence of bone growth in the control group. Hu et al.¹⁶⁸ examined TiO₂ nanotube arrays loaded with BMP-2 that was covered with multilayered coatings of gelatin/chitosan (Gel/Chi). The multilayered coatings were employed for controlling the release of BMP-2. This system showed increased osteoblastic differentiation of mesenchymal stem cells.

Among other polymers, hyaluronic acid (also called hyaluronan), which is a polyanionic polysaccharide, is commonly used in implant research and has structural characteristics similar to chitosan¹⁷³. Some reports have shown that hyaluronic acid coating on metallic implants has excellent lubricity of a non-adhesive nature and positive effect on chondrocyte and osteoblast activity¹⁷⁴⁻¹⁷⁶. Hyaluronic acid was proven to have bacterial inhibitory effects^{174, 177}. Thus, it is used for different applications, such as wound healing processes suitable to combine tissue engineering, and drug delivery carriers¹⁷⁸⁻¹⁸⁰. Hyaluronic acid was examined for bone regeneration as scaffold materials with the addition of rhBMP-2 and showed improved tissue remodeling rates and high mature bone formation^{181, 182}. Furthermore, it was frequently examined with dental implants to enhance the bone response in combination with bone stimulating agents^{183, 184}. For instance, hydroxyapatite-coated porous titanium with BMP-2 and hyaluronic acid were placed in femoral metaphyses of rabbits to assess their osseointegration capability¹⁸⁵. The histological analysis showed more newly formed bones in the BMP-2 and hyaluronic acid group compared to the HA-coated titanium group. Moreover, hyaluronic acid hydrogel can be easily applied with bone graft materials to repair and reconstruct bone defects or to fill gaps between alveolar bone and dental implants¹⁸⁵⁻¹⁸⁷.

Poly-(lactic-co-glycolic acid) (PLGA)

Poly-(lactic-co-glycolic acid), better known as PLGA, is a biodegradable copolymer with high biocompatibility that has been extensively investigated in pharmaceutical and biomedical applications¹⁸⁸⁻¹⁹⁰. The degradation behavior of PLGA was reported

to allow sustained drug release at the desirable doses ¹⁹¹. In addition, the rate and duration of drug release can be modified according to the obtained degradation behavior. Naito et al.¹⁹² examined the release profile *in vitro* of simvastatin from PLGA that was synthesized as microspheres of about 20 µm in size. The degradation behavior of the microspheres helped in slowing down the release of simvastatin from PLGA, which lasted from approximately 2 weeks to more than 1 month. The drug release from PLGA microspheres was characterized by a burst release in the first days followed by bulk diffusion from the microsphere as the degradation process of microspheres continued ¹⁹³. These findings were also observed in other studies that investigated the incorporation of bone stimulating agents into PLGA microspheres loaded with bone substitutes ^{192, 194}.

In a recent study, implant surfaces consisting of titania nanotubes were coated with PLGA to evaluate the release profile of ibuprofen from the coatings ¹⁹⁵. Different thicknesses of PLGA were formed on the implant surface. Subsequently, the PLGA coating revealed sustained release of ibuprofen up to 40 days with significant decrease in burst release from 84% to 27%. Changing the thickness of PLGA coatings allowed for control over the ibuprofen release behavior.

Poly (N-isopropylacrylamide) (PNIPAAm)

Some polymers demonstrate dramatic physical and volume changes in response to external stimuli, such as temperature, pH, and light ¹⁹⁶⁻¹⁹⁹. Thus, these materials were found having significant potential for different applications, including drug delivery.

Thermoresponsive polymers are a popular class of materials that can respond to changes in temperature ^{200, 201}. Among these, poly(*N*-isopropylacrylamide) (PNIPAAm) has frequently been studied for tissue engineering and drug delivery applications, because of its unique thermal properties ^{200, 202, 203}. PNIPAAm has a unique feature of responding rapidly to changes in temperature, causing a phase transition. This phase transition occurs at temperature known as the lower critical solution temperature (LCST) around 32-34 °C ²⁰². During the phase transition, PNIPAAm exhibits a conversion from a

swollen and hydrophilic state (below LCST) to a collapsed and insoluble (hydrophobic) state above LCST (Figure 4) ²⁰⁴.

Figure 4. Illustration of the volume phase transition of PNIPAAm from a swollen and hydrophilic state below the lower critical solution temperature (LCST) to become collapsed and hydrophobic above the LCST.

At a temperature below LCST, a PNIPAAm hydrogel can be loaded with drugs and at a determined point be subjected to heat to display and release its contents. The hydrogel can be designed to show fast response to thermal stimuli with promising potential for controlled drug release²⁰⁵. The temperature sensitivity demonstrated by PNIPAAm has attracted attention in several research projects. For instance, Kun Na et al.²⁰⁶ examined a hybrid scaffold made of hydroxyapatite and PNIPAAm-co-AAc as an injectable vehicle containing BMP-2. The scaffold improved the osteogenic differentiation of mesenchymal stem cells and homogeneous bone formation throughout the scaffold. In another study, an *in vivo* experiment was performed to evaluate an injectable hydrogel composed of poly(NIPAAm-co- acrylic acid) developed to support tissue formation. The poly(NIPAAm-co- acrylic acid) was histologically examined and revealed good potential for tissue engineering applications²⁰⁴.

Poly(N-isopropylacrylamide) (PNIPAAm) can be designed as substrates to provide a suitable environment for cell proliferation and to release culture sheets²⁰⁷. In addition, PNIPAAm can be

created as a degradable artificial matrix to enable cell proliferation and migration in three dimensions ²⁰⁸. A lab and animal experiment reported by JH Cho et al.²⁰⁹ showed that an injectable material formed using PNIPAAm and water-soluble chitosan (WSC) copolymer could be used to differentiate mesenchymal stem cells (MSCs) into chondrocytes. These experiments in addition to other studies, suggest that thermoresponsive surfaces or matrixes based on PNIPAAm can be recognized as powerful tools for tissue engineering and cell proliferation and differentiation^{207, 208, 210}.

For drug delivery applications, the release mechanisms of drugs loaded in PNIPAAm polymers can be controlled by a cyclic swelling and shrinkage behavior above and below the LCST ²¹¹. Thus, these polymers can be described as a pump that imbibes the drug on cooling and releases the drug on heating. Drug release from temperature sensitive polymers such as PNIPAAm and its copolymers occurs by Fickian diffusion during polymer shrinking ²¹¹. However, drug release by this mechanism occurs also when the polymers are on their swollen state ²¹².

A supporting matrix has been synthesized using poly(N-isopropylacrylamide-co-acrylic acid) hydrogel for cartilage repair and cell therapy ²¹³. The matrix was incorporated with growth factors in the form of ascorbate and dexamethasone and showed improved cell differentiation compared with the control matrix without drugs. Bridges et al. in 2008 and 2010 investigated in lab and animal experiments a thin film of microgels composed of poly(N-isopropylacrylamide)-co-acrylic acid and cross-linked with poly(ethylene glycol) diacrylate^{214, 215}. This coating material was utilized as a protective layer on implant surfaces. The formation of the microgel coating was performed on a substrate using spin coating technique. The *in vitro* and animal tests of the microgel coating showed significant reduction in leukocyte adhesion and activation. Therefore, these results suggest that the microgel coating can be applied to modulate acute and chronic inflammation responses during implant application.

Using the spin coating technique, an ultra-thin layer of PNIPAAm (up to few nanometers) was easily made. This very thin layer was

formed by copolymerization with a vinyl monomer and was cross-linked and immobilized by heat treatment ²¹⁶.

A copolymer composed of *N*-isopropylacrylamide (NiPAAm):*N*-*tert*-butylacrylamide (NtBAAm) polymers has been used to form film layers that can be applied to deliver therapeutic agents to vascular smooth muscles^{217, 218}. With the goal of evaluating drug release properties, the copolymers were incorporated with an antimitotic agent and vascular endothelial growth factors. The evaluation of the release profile showed the ability to regulate and prolong the drug release over a period of time that could be useful in preventing and repairing vascular diseases.

PNIPAAm with incorporated gold nanorods (PNIPAAm-GNRs): For potential clinical applications, a local heating source is required to obtain the phase transition of PNIPAAm. This is necessary to control the drug release. One of the suggested methods involves incorporating gold nanoparticles into the polymer. Interestingly, gold nanoparticles with particular designs, e.g., nanoshell and nanorods, can absorb light intensively in the near infrared (NIR) spectrum and convert the light energy to heat ²¹⁹⁻²²². Thus, irradiation with NIR can be utilized as external stimuli to generate the heat needed to achieve the phase transition of PNIPAAm. The intrinsic body components such as water have low optical absorption of light in the NIR region (650–900 nm), allowing deep penetration of light in the tissue ²²³. Shiotani et al. ²²⁴ reported that the PNIPAAm-GNR composite can rapidly shrink using NIR light compared with direct heating. In their experiment, homogenous incorporation of gold nanorods in the polymer gel was achieved. After irradiation with NIR, rapid shrinkage and release of the model drug (rhodamine-labeled dextran) was observed. As recommended for providing the required gel shrinkage to release the drug, it has been suggested that gold nanorods should not have concentration less than 100 μ M when the irradiation power is between 490 and 750 mW²²⁴.

Other studies have examined the synthesis of PNIPAAm as microgels loaded with GNRs to investigate the temperature-induced volume phase transition with NIR irradiation ^{220, 225}. Due to their

small sizes, microgels have rapid phase transition response to increased temperature, allowing targeted drug delivery. When a drug is loaded in the microgel, the shrinkage behavior can cause the microgel to release the drug ²²⁶.

Mallika et al.²²⁵ investigated PNIPAAm microgels loaded with GNRs that were synthesized with various monomer compositions. The aim was to obtain a phase transition behavior above the physiologic body temperature (37°C). Among different compositions that were examined, poly(N-isopropylacrylamide-maleic acid) succeeded to show suitable de-swelling properties at a temperature range of 38-41°C. Furthermore, Poly(N-isopropylacrylamide-acrylic acid) [poly(NIPAM-AA)] microgel was formed with a particle diameter around 500 nm ²²⁰. The microgels were doped with gold nanorods that were designed to absorb NIR light. After being subjected to many laser on/off cycles, the microgels showed repeated de-swelling and swelling responses, opening the doors for targeted delivery of small drug molecules.

Assessment of drug release

Assessment of drug release behavior can offer important information about the efficacy of the drug delivery system. *In vitro* release tests are commonly used to eliminate the need for human/animals and to overcome costs and time limitations associated with *in vivo* measurements. In addition, the *in vitro* release tests are usually aimed to investigate the release mechanism and kinetics²²⁷. Such tests can sometimes provide information on the delivery system structure (e.g., porosity) and its effect on the rate of drug release ²²⁸. This information is very important in developing a drug delivery system with predetermined properties such as a sustained drug release. For potential clinical application, the *in vitro* tests required to predict the *in vivo* drug release as much as possible. The tests should consider the physiological variables such as body temperature and metabolism. Evaluation of the release should be performed directly from the formed carrier materials such as injectable biodegradable microspheres and implants ^{229, 230}.

Proper *in vitro* tests should be able to detect a high initial release (burst release) from the carrier ²³¹. Obtaining information about

burst release and its duration is important to evaluate the influence of the drug release profile on the *in vivo* efficacy and safety window²³².

Many drug release tests do not offer immediate analysis and need predetermined solution concentration measurements as function of time to be performed^{233, 234}. This could result in inaccurate measurements of drug dissolution and release^{235, 236}. However, some imaging techniques can be used to deliver spatial and temporal information for drug release mechanisms. These imaging techniques include magnetic resonance imaging (MRI)²³⁷, electron Paramagnetic Resonance (EPR) spectroscopy²³⁸, and NMR spectroscopy²³⁹. Meanwhile, other techniques such as UV/VIS spectroscopy and QCM-D are suitable to study the *in vitro* release of drug delivery system formed as implant coating materials^{154, 157, 158, 240, 241}. These techniques have shown to be particularly useful for providing real-time information of early events during drug dissolution and release. In addition, the analysis can be performed using a small amount of drug.

Some techniques used for *in vitro* release tests

UV/VIS spectroscopy: UV/VIS spectroscopy involves the absorption of radiation in the ultraviolet-visible spectral region. Ultraviolet light has wavelengths between 190 and 400 nm, while the wavelengths for visible light are between 400 and 800 nm. During the UV/VIS spectroscopy analysis, the light intensity at a particular wavelength passes through a quartz cell to create UV imaging. This imaging is based on the absorbance (transmittance) of UV light as a function of position and time²⁴². The mechanism of UV/VIS spectroscopy work is based on Beer-Lambert's law that describes a direct proportional between absorption, solution concentration, and path length. Thus, the absorbance measurements allows for *in situ* quantification of the detected drug substances. In addition, concentration gradients obtained from the drug delivery system can be recorded in real time²⁴². UV/VIS spectroscopy can be employed for accurate and precise analysis of drug dissolution and release processes as they occur in the vessel. Interestingly, many drugs absorb light in the UV spectral range. Measurements using UV/VIS

spectroscopy is believed to be simple, cost-effective, and can be performed using small volumes. Today, UV/VIS spectroscopy is commercially available and considered one of the most important tools for drug analysis. However, one of the limitations using UV/VIS spectroscopy is its low sensitivity for some drugs. The sensitivity of a UV/VIS spectroscopy can also be low at low sample concentrations. Meanwhile, the absorption results can be influenced by different factors such as temperature, pH, and impurities in the sample that reflect light²⁴³. These factors can influence the absorption properties of the sample leading to an erroneous reading.

Quartz crystal microbalance with dissipation monitoring (QCM-D): QCM-D is a very sensitive nano-scale technique employed to obtain real-time information of the adsorbed mass, such as a thin film formation on flat substrates. The QCM-D technique is based on a piezoelectric quartz crystal oscillation to monitor frequency changes (Δf) based on the oscillating mass of the sensor and adhering films. Rodahl et al.²⁴⁴ have previously described the principles of QCM-D with some detail. When a rigid film is evenly deposited onto the QCM-D sensor crystal, a proportional decrease in resonant frequency (Δf) is observed according to the film mass (Δm). The Sauerbrey equation can be used to measure the change in film mass according to the change in frequency as follows²⁴⁵:

$$\Delta m = (-C \times \Delta f) / n$$

In this equation, Δm represents the mass change, Δf represents the frequency change (Hz), n represents the frequency overtone, and C represents the mass sensitivity for a 5-MHz crystal that is known to be 17.7 ng/(cm² Hz). If the adsorbed film is not rigid, the oscillation of the sensor results in energy dissipation (ΔD). The dissipation can be measured by monitoring the response of oscillating when the sensor vibrates at its resonance frequency, and this method was employed to evaluate the viscoelastic properties of the adsorbed film. Thus, the QCM-D is suitable for studying surface-related

conformational changes ^{246, 247}, including evaluation of drug absorption and release ¹⁵⁷.

Some studies has revealed that QCM-D analysis sometimes under or overestimates the adsorbed mass when compared with other techniques like AFM ^{246, 248, 249}. In QCM-D analysis, the calculated mass can be attributed to coupled water and not only to the viscoelastic properties of the film ²⁵⁰. The frequency changes detected with QCM-D needs to be carefully interpreted since the influence of the mass increase cannot be differentiated from that of water ²⁵¹.

This literature review found particularly interesting results for using local drug release to enhance the bone response around implants. Local drug release has been identified as a promising strategy for administration of different kinds of therapeutic agents ^{157, 252, 253}. The drug release at the implant site could provide a direct delivery of drug agents to the bone-implant interface ^{254, 255}, and may be beneficial to the patients since it reduces the risks sometimes observed with systemic administration ^{122, 256}. For endosseous implants, different methods were investigated for local drug release such as coating of implants with drugs and chemical compounds ^{120, 121}. Meanwhile, many studies have reported sustained drug release from implants covered with mesoporous TiO₂ films ^{157, 257}. Drug release using carrier materials is another common method that has been examined numerously for local drug release around implants ^{258, 259}. Polymeric materials like PLGA and PNIPAAm were found interesting as drug carrier materials. Some studies showed that these polymeric materials allow sustained drug release for a prolonged period of time ^{218, 260, 261}. Therefore, one aim of the thesis was to map out the different methods used for local drug delivery to implants sites, and whether these methods could enhance bone formation. Furthermore, to examine the use of mesoporous TiO₂, PLGA, and PNIPAAm as local drug delivery systems.

AIMS

(Systematic review)

- To map out the methods most commonly used for local drug and chemical compound delivery to implant sites and assess their influence on bone response.

(In vitro)

- To evaluate a new developed surface coating designed for medical devices such as implants, and to examine the drug release mechanism from the coating using near infrared light (NIR) as an external stimulus.
- To study *in vitro* the release of clarithromycin from PLGA microspheres within beta-tricalcium phosphate (β -TCP).
- To evaluate the local release of strontium ranelate from implant surface coated with mesoporous titania films.

(In vivo)

- To evaluate the osteogenic effect of clarithromycin-loaded microspheres incorporated into β -TCP cement in a calvaria defect model *in vivo*.
- To examine if the local release of Sr-ranelate could improve bone formation around implants in an animal model.

MATERIALS AND METHODS

Data collection (Systematic review)

Search protocol: In the systematic review (Study I), an electronic search was performed to find publications evaluating the use of drug and chemical compound delivery systems together with endosseous implants in animal studies. The electronic search was done through three databases: PubMed, SCOPUS, and Embase. In addition, the reference lists of the studies and the reviews on the subject were also checked for any relevant publication. The search was undertaken in November 2016 without time restrictions and publications written in English were only evaluated. The search strategy for each database was related to three main components; bone, implant, drug and chemical compound delivery. The following keywords were used to search for publications:

("bone remodeling" OR "bone regeneration" OR "bone development" OR "bone growth" OR "osseointegration") AND ("biocompatible coated materials" OR "endosseous dental implantation" OR "dental implants" OR "implants, experimental" OR "bone-implant interface") AND ("diffusion" OR "drug compounding" OR "drug implants" OR "drug delivery" OR "drug delivery device" OR "drug delivery system" OR "drug release" OR "local drug delivery") AND ("animal experimentation" OR "animal models")

Inclusion criteria: The main criteria for publications to be included in this systemic review was to report the use of endosseous implants combined with a therapeutic agent or chemical compound evaluated using animal studies. The therapeutic agent (drugs, proteins, growth factors, antibiotic, etc.) or the chemical compound should be administrated locally or released from the implant surface. Loading of these agents and compounds were supposed to be done either before or during implant insertion.

Data extraction: For the study selection process, abstracts of all identified studies were read independently by two authors for initial screening. After screening, the studies judged to meet the inclusion criteria were read completely in addition to other studies that showed insufficient data in the title and abstract. Disagreements were resolved by discussion between the authors. From the publications collected in the last evaluation stage, different data were extracted on a standard form. This data include; type of drug or chemical compound, the drug delivery system, the animal model, number of animals, number of implants, time period between the surgery of implantation and the euthanasia of the animals, and mean ($\pm$ standard deviation) values of bone-to-implant contact (BIC).

Principles of the new developed drug delivery system

In Study II, we designed a drug delivery system using a thin GNR-containing PNIPAAm coating and NIR light source as an external stimulus (Figure 5). In this system, GNRs were used for heat generation since it has been shown in numerous reports to have the ability to absorb light intensively in the near infrared (NIR) spectrum and convert it to heat ²¹⁹⁻²²². The generated heat was observed to be enough to increase the PNIPAAm temperature above LCST. Thus, GNRs can induce PNIPAAm shrinkage to obtain drug release ²²⁴. The designed drug delivery system contained double layers of PNIPAAm in which the GNRs are distributed in between the layers. This was suggested to maximize the thermal effects of GNRs and to avoid a direct contact between tissue and GNRs in a

clinical setting. The polymer surface will be supported by mesoporous TiO_2 film to improve drug-loading capacity.

Figure 5. Schematic illustration of the local drug delivery system.

Materials preparations

PNIPAAm Polymers synthesis

In Study II, PNIPAAm was synthesized as homopolymer (the conventional PNIPAAm, polymerized using single monomer: *N*-Isopropylacrylamide) or co-polymerized with acrylamide at 5% and 10% weight ratios.

Used materials: *N*-isopropylacrylamide (NIPAAm) and acrylamide monomers were purchased from Sigma. *N,N'*-Azobisisobutyronitrile (AIBN) as initiator was kindly provided by the laboratories at the Department of Chemistry and Chemical Engineering at Chalmers University of Technology. 2-Hydroxy-4-(methacryloyloxy)benzophenone, 99%) as photo-cross-linking agent was purchased from Alfa Aesar.

PNIPAAm: 1 g of NIPAAm monomer was dissolved in hexane prior to use. PNIPAAm polymer was synthesized by free-radical polymerization using 0.0354 g of AIBN (mole ratio 1:41 to monomer), and 20 mg of 2-hydroxy-4-(methacryloyloxy) benzophenone (weight ratio 2% to monomer). In brief, the reactants first were dissolved in 1 ml of ethanol, and then kept under gentle stirring for 15 min. Later, the obtained solution was transferred to a flask with a septum and purged with nitrogen for 5-10 minutes to remove oxygen. The flask was then immersed in an oil bath at 60°C for 60 minutes. Following that, the flask was opened to air to terminate the reaction. The obtained polymer was then stored in the fume hood overnight at room temperature. Later, The polymer was washed with milli-Q water numerously to remove any unreacted excess reagent.

PNIPAAm-AAm; Poly(N-isopropylacrylamide) copolymerized with acrylamide (PNIPAAm-AAm) was synthesized using same synthesis procedures mentioned with PNIPAAm plus the addition of 50 and 100 mg of acrylamide (5% and 10% weight ratio to NIPAAm).

PLGA microspheres loaded with Clarithromycin

In Study III, clarithromycin was loaded into PLGA microspheres to obtain sustained drug release.

Used materials: Poly (lactic-co-glycolic acid) (PLGA), Polyvinyl alcohol (PVA), dichloromethane (DCM), acetonitrile, ethanol and acetone were used for the microsphere synthesis (Wako Pure Chemical Industries, Osaka, Japan). Clarithromycin used as a drug model was provided by Taisho Pharmaceutical (Tokyo, Japan).

Clarithromycin-loaded PLGA microspheres preparation

PLGA microspheres were prepared by modified O / W single emulsion / solvent evaporation method. This method involved 10 mg clarithromycin and 500 mg PLGA dissolved into 3 mL of DCM. The mixture was added into 300 mL of 0.25% (w / v) PVA solution and homogenized at 10,000 rpm (Polytron, Kinematica AG, Luzern, Switzerland) for 3 min at room temperature. Later, it was stirred for

6 hours to remove DCM and allow solvent evaporation. The microsphere suspension was centrifuged at 6,000 rpm for 5 min. Following, the microspheres were washed vigorously in distilled water then frozen to -80 °C for 30 min. They were later freeze-dried for 24 hours using a freeze dryer (FD-1; EYELA, Tokyo Rikakikai, Tokyo, Japan).

Mesoporous titania film formation

In Studies II and IV, a uniform thin layer of mesoporous titania was formed following the technique described elsewhere ¹⁵⁴. In brief, a titania precursor solution was first prepared by mixing 2.1 g titanium (IV) ethoxide (20%) with 1.6 g concentrated hydrochloric acid (37%). The mixture was placed under vigorous stirring to form a homogeneous solution. Pluronic® P123 (triblock copolymer EO20PO70EO20, 0.5 g) was dissolved in 8.5 g ethanol with vigorous stirring followed by mixing with the precursor solution. The final solution was left to stir for approximately 24 h to achieve a homogenous mixture. To obtain uniform thin films of mesoporous titania, 80 µl of the final solution was spin-coated on glass slides (1x1 cm), titanium screws, and Ti-coated QCM-D sensors at 4,000 rpm for 60 sec using spin coater. The coated surfaces were left for 1 day at room temperature to obtain complete the self-assembly. Finally, the films were calcinated by heating for 5 hours with a heating ramp of 1°C/min to 350°C. The films were then left at temperature 350°C for 4 hours to remove the template and to cross-link the titania.

In Study IV, a second mesoporous titania film P123-PPG was prepared following the same procedure as previously described plus the addition of poly propylene glycol (PPG) to P123 (1:1 by weight) to enable the formation of larger pore size ²⁶².

Gold nanorods (GNRs)

In Study II, GNRs were incorporated into the PNIPAAm layer and utilized for local heat generation to enable elevating the PNIPAAm temperature above LCST.

Used materials: Bare gold nanorods solution in CTAB was purchased from Nanopartz Inc. (CO, USA). Hexamethyldisilazane,

poly(ethylene glycol), average M_n 6,000, was purchased from Sigma Aldrich.

PEG-coated GNRs: The GNRs stabilized by CTAB were modified with poly(ethylene glycol) (PEG) to exchange the CTAB molecule. This was reported as an effective approach to improve the biocompatibility and dispersibility of the rods ²⁶³. A solution of gold nanorods containing CTAB was centrifuged at 3000 rpm for 40 min, decanted, and resuspended in milli-Q water to remove excess CTAB. A total of 1 ml of GNRs solution (2.8 nM) was added to 200 μ l of 5 mM PEG (M_n 6,000). The mixture was stirred at room temperature overnight. The resulting mixture was dialyzed the next day to remove any unreacted PEG then stored in a refrigerator (- 4 °C).

Characterization of materials

Differential scanning calorimetry (DSC)

In Study II, the thermal behavior of the PNIPAAm and PNIPAAm-AAm polymers was determined using differential scanning calorimetry (DSC). The calorimetric analysis was carried out on a Perkin-Elmer differential scanning calorimeter. Before DSC measurements, all the polymer samples were immersed in milli-Q water for 1 day at room temperature allowing them to swell to equilibrium. For each DSC measurement, around 5 mg of the swollen samples were sealed tightly in aluminum pans. The thermal analysis was performed for the samples from 22°C to 45°C under a dry nitrogen atmosphere at a heating rate of 1°C /minute. Dehydration of the aqueous PNIPAAm-based polymer was suggested to occur with increasing temperature and combined with a clear thermal effect, thus, an endothermic peak can be observed on DSC curve ^{264, 265}. The LCST is defined by the point between the moment of increase in the DSC endotherm and the maximum of the DSC peak.

Scanning electron microscopy (SEM)

In Study II, top and cross-sectional images of the mesoporous titania films and the polymer coating were obtained using a Leo Ultra 55 electron microscope (Carl Zeiss Meditec AG, Oberkochen, Germany)

operating at 2-5 kV. In addition, the mesoporous titania pore size (Studies II and IV) and film thickness (Study II) were measured.

In Study III: the surface morphology and particle size of the microspheres was visualized by scanning electron microscopy (SEM; S-4300, Hitachi High-Technologies Corporation, Tokyo, Japan). The size of the microspheres was calculated by measuring the feret diameter.

Mechanical test: In Study II, the PNIPAAm layer stability and resistance to detachment were examined using titanium implant screws. The implant screws were coated with PNIPAAm similar to the procedures previously described. Then, the screws were inserted into pre-drilled holes in animal bone similar to the standard implant insertion steps made on humans. Finally, the screws were removed from the bone and examined with SEM to assess the coating condition.

UV/VIS spectroscopy

In Study II, absorption spectra of GNRs stabilized with CTAB and GNRs modified with Poly(ethylene glycol) (PEG) were measured using UV/VIS spectroscopy (Agilent 8453, USA). This was done to examine the absorbance peak of the GNRs from the visible to near-infrared light region. In this experiment, an LED lamp was used as an NIR light source with a maximum intensity of 1 (W/cm²) at 850 nm.

In addition, the UV/VIS spectroscopy was used to evaluate the drug release behavior from PNIPAAm-coated surfaces upon irradiation with NIR. For this test, phenol was selected as the drug model. PNIPAAm-coated glass slides (with or without GNRs) were immersed in a phenol/water solution (0.5 g/ml) for a full day to obtain good saturation with phenol. The glass slides were then washed with milli-Q water and dried with nitrogen gas. Later, the coated surfaces were placed in the bottom of UV/VIS cuvettes containing 2 ml of milli-Q water. One cuvette was filled only with milli-Q water to be used as a blank. The absorbance values were measured at 269 nm wavelength since phenol demonstrates a peak

at this wavelength. For the analysis, the samples (n=4 /group) were irradiated with NIR light for 30 minutes in which the absorbance values were followed regularly before and after irradiation to evaluate the release kinetics from the samples. The standard calibration curve of the absorbance as a function of the phenol concentration was obtained at 269 nm to determine the exact phenol concentration during the analysis. Analyses are presented as mean values.

In Study III, UV spectrophotometry was used to measure clarithromycin release behavior from PLGA microspheres in phosphate-buffered saline (PBS). The concentration was calculated at a wavelength of 261 nm. PBS solution containing PLGA microspheres was placed in a dialysis tube (Spectra/Pore membranes MWCO 12–14,000) with 3 mL of PBS suspended in 500 ml of the same medium under gentle stirring at 37°C. The dissolution medium outside the dialysis tube was collected, filtered then replaced with fresh PBS after each time period. The amount of clarithromycin was determined at 1, 2, 3, 5, 7, 14, 21, and 28 days.

Quartz crystal microbalance with dissipation monitoring (QCM-D)

In Study II and IV, QCM-D analyses were performed using the Q-Sense E4 model (Q-Sense, Sweden). The principles of QCM-D were described in detail by Rodahl ²⁴⁴.

In Study II, QCM-D analysis was used to calculate the accessible pore volume on PNIPAAm-coated QCM-D crystals following a technique described elsewhere²⁶⁶. In brief, an 80 µl of PNIPAAm dissolved in ethanol (10% concentration) was spin coated on gold-coated QCM-D crystals. Uncoated QCM-D crystals were used as a control. The pore volume in PNIPAAm coating was calculated by the water exchange between milli-Q water (H₂O) and pure deuterium oxide (D₂O) using the Sauerbrey equation as previously described in the introduction ²⁴⁵.

The QCM-D was also used in Study II to evaluate the frequency and dissipation changes that the PNIPAAm layer on Ti-coated crystal demonstrated in response to NIR irradiation. Before the analysis, PNIPAAm-coated QCM-D crystals (with or without GNRs)

were placed in QCM-D chambers while having a flow of milli-Q water for 30 min. The analysis started at room temperature for 60 minutes to obtain a stable baseline before NIR irradiation. Thereafter, the PNIPAAm-coated crystal was irradiated with NIR light for 30 minutes. The changes in the frequency and dissipation for each crystal were registered at the 3rd overtone.

In Study IV, the QCM-D was used to evaluate the amount of Sr-ranelate uptake and release from the mesoporous TiO₂ surfaces. The uptake and release behavior was monitored following the change in frequency (Δf , Hz). The change in frequency can be employed to calculate mass differences (Δm , ng/cm²) using the Sauerbrey equation ²⁴⁵. Prior to the analysis, spin-coating was carried out to deposit mesoporous titania coatings made of P123 and P123-PPG templates onto Ti quartz crystal sensors (Q- Sense, Sweden). During the analysis, an initial flow of milli-Q H₂O was obtained at constant flow rate of 50 ml/min to reach a stable baseline. Afterward, a flow at 50 ml/min of Sr-ranelate dissolved in milli-Q H₂O (1 wt% strontium) was added to monitor the drug absorption into the mesoporous film. The Sr-ranelate was then exchanged with flow of milli-Q H₂O only (50 ml/min) to evaluate the Sr-ranelate release from the mesoporous film. The obtained mass values were used to calculate the amount of Sr-ranelate absorption and release (ng/cm²).

Bacterial test

In Study II, the bacterial growth around PNIPAAm-coated surfaces loaded with vancomycin was assessed using *Staphylococcus epidermidis* (ATCC 35984). In this experiment, glass substrates coated with mesoporous titania and PNIPAAm were placed in set of Petri dishes inoculated with *Staphylococcus epidermidis*. Three groups of PNIPAAm-coated glass were prepared;

Group 1 (Control)	Substrates coated with mesoporous titania and PNIPAAm loaded with vancomycin.
Group 2 (Control)	Substrates coated with mesoporous titania and GNRs-incorporated PNIPAAm.
Group 3 (Test)	Substrates coated with mesoporous titania and GNRs-incorporated PNIPAAm loaded with vancomycin.

Before the experiment, the glass substrates were cleaned by treatment in a hot basic piranha solution (1:1:5 ammonium hydroxide, hydrogen peroxide, and deionized water) for 20 minutes at 75°C. Then, the substrates were washed thoroughly with milli-Q water and dried with nitrogen gas. The mesoporous films and the polymer coatings were formed using the same techniques described before. The substrates in groups 1 and 3 were loaded with vancomycin using the immersion technique in which it were kept in vancomycin solution (0.2 mg/ml) for 24 hours to allow good saturation of the drug. Thereafter, the substrates were removed and dried gently with nitrogen gas.

Bacterial culture: A sterilized 10 ml loop was used to grow a single colony from the culture agar plates of each bacterium to inoculate a tube of 5 ml tryptic soy broth. The inoculated cells were cultured in the incubator for 6 h, then diluted in tryptic soy broth thereafter left in the incubator overnight to allow the bacterial growth to a stationary phase. Afterward, part of the suspension was plated onto agar casted in Petri dishes and then placed in substrates with the coating pointing towards the agar surface. For each group, 3 samples were prepared and placed in a separate Petri dish. Bacteria were cultured under the standard culture conditions (5% CO₂/95% air at 37°C) to support biofilm formation onto the surfaces.

Bacterial growth and inhibition zones: The substrates were irradiated for 10 minutes with NIR light with a maximum intensity of 1 W/cm² at 850 nm. All the substrates were imaged before and after NIR irradiation to determine bacterial growth and if bacterial inhibition zones existed.

In addition, the antimicrobial behavior of GNRs upon NIR illumination was evaluated without the presence of the antibiotic. *Staphylococcus epidermidis* was cultured directly onto the GNRs-incorporated PNIPAAm coated onto glass substrates (1 × 1 cm). Later, the substrates were irradiated with NIR for 10 minutes followed by staining with BacLight LIVE/DEAD (Thermo Fisher Scientific) and examined using fluorescence microscopy.

In vivo methods

Ethical approvals: The animal surgeries in Studies III and IV were performed under the approval from the ethical committee for animal experiments at the Ecole Nationale Veterinaire D'Alfort, Masion D'Alfort, France.

Animal model: Twenty-one New Zealand rabbits were used in Study III. While for Study IV, twenty-four 6-month-old Sprague Dawley female rats were used.

Surgical procedures

In Study III, the rabbits were anesthetized using ketamine chlorate (15 mg kg⁻¹) then the rabbits head were shaved and disinfected by iodine solution. Later, a flap was raised to create four bi-cortical bone defects (5 mm of diameter) in the calvaria bone in each rabbit. The defects were randomly divided into four groups:

Group 1	No augmentation (sham).
Group 2	Beta-Tricalcium phosphate (β-TCP).
Group 3	β-TCP with 0.12 mg clarithromycin.
Group 4	β-TCP with 6.12 mg PLGA microspheres (0.12 mg clarithromycin).

The animals were euthanized with anesthesia overdose after 2, 4, and 12 weeks of healing. At each time point, seven rabbits were used (21 rabbits in total).

In Study IV, the rats were anesthetized through inhalation of isoflurane 1% dissolved in O₂. Access to the tibia was gained by an incision in the skin over the medial face of the tibia followed by elevation of full-thickness periosteal flap to expose the medial tibia plate. Drilling of the implant site was conducted with a sequence of 1-mm- and 2-mm-diameter burs, under constant irrigation of saline solution. Mini-screws implants coated with mesoporous TiO₂ were inserted in both sides randomly following four experimental groups:

Group 1	Mini-screws coated with P123
Group 2	Mini-screws coated with P123-PPG
Group 3	Mini-screws coated with P123 and loaded with Sr-ranelate.
Group 4	Mini-screws coated with P123-PPG and loaded with Sr-ranelate.

Two screws were inserted in each tibia, with a total of four screws per animal, two tests and two controls (Figure 6). The animals were euthanized after 2 and 6 weeks by pentobarbital overdose.

In Studies III and IV, after animal euthanasia, the specimens were removed en bloc and fixed in 4% formaldehyde for 24 h. subsequently, the samples were dehydrated in a series of ethanol concentrations (70–100%) and infiltration in resin under constant vacuuming. The samples were then embedded in light curing- resin (Technovit 7200 VLC; Heraeus Kulzer Wehrheim, Germany).

Figure 6. Descriptive surgical procedures showing: (A) A flap was raised and the implant site was prepared using a sequence of 1 mm and 2 mm diameter burs. (B) Implants placement with 2 mm distance between the screws.

Micro-computed tomography (μ CT)

In Study III, a 3-dimensional bone formation in the defect was evaluated using micro-computed tomography (μ CT, SkyScan 1176, Bruker Micro-CT, Kontich, Belgium). The measurements of the newly formed bone volume in the defect area were performed using imaging software (Data Viewer, CT-Analyser, Bruker Micro-CT, Kontich, Belgium). The image files were first opened and calibrated in the software. For the measurements, the defect area was selected, thereafter; all pixels within this area with grey value intensities over a predetermined threshold that corresponded to the mineralized bone was calculated as a newly bone formation volume. The percentage of newly formed bone in each defect area was calculated by calculating the newly bone formation volume (μm^3) divided by the total defect volume (μm^3). The measurements were presented as mean values $\pm$ standard error.

Preparation of histological sections

In Study III and IV, the resin-embedded samples were subjected to undecalcified ground sectioning to obtain 100 μm -thick sections using the Exakt sawing and grinding equipment²⁶⁷. Later, the sections were ground to a final thickness around 20 μm and stained with Toluidine blue-pyronin dye for the histologically analyses.

Histomorphometry

For histomorphometry, the stained sections were evaluated using a light microscope (Eclipse ME600—Nikon Co., Tokyo, Japan). In Study III, a region of interest (ROI) representing the defect area was selected on each histological slide. The amount of new bone formed within the ROI was calculated as percentage (NB%) over the total area of the ROI. In Study IV, the following measurements were calculated; the percentage of bone-implant contact (BIC), the percentage of bone area between threads (BA), and the percentage new bone area (new-BA) between threads. The histomorphometric analyses for the two studies (Studies III and IV) were performed using the Image J software (National Institutes of Health, Bethesda, MD, USA).

Statistics

In the systematic review (Study I), Bone-Implant Contact % (BIC) was the continuous outcome evaluated. Weighted mean differences were used to construct forest plots. Number of implants used for the histological analysis for each group was the selected statistical unit. Three groups of drug/chemical compounds were evaluated; bone morphogenetic proteins (BMP), bisphosphonates (BP), and calcium-phosphates (CaP). Two groups for methods used in local drug delivery were also evaluated. These methods were either by drug delivery directly from the implant surface or by local administration to the implant site. All studies classified by drug type or delivery method were analyzed in total, by subgrouping them according to animal kind (rabbits, dogs, rats, pigs, and goats), and by time periods between the surgery of implantation and the euthanasia of the animals. If the BIC% was not clearly stated, the data were not used for analysis. The I^2 statistic was used to express the percentage of the total variation across studies due to heterogeneity. The inverse variance method was used for random-effects, when there was statistically significant ($P < 0.10$) heterogeneity, or fixed-effects model, for when heterogeneity was not statistically significant²⁶⁸. The estimates of an intervention were expressed in mean difference (MD) in percentage, with a 95% confidence interval (CI). The software Review Manager (version 5.3.3, The Nordic Cochrane

Centre, The Cochrane Collaboration, Copenhagen, Denmark, 2014) was used.

In Study III, the statistical analyses were performed with SPSS (version 18, Chicago, IL, USA). The Friedman rank test was used to assess the differences between all groups during the three time points. The test was followed by post hoc test for multiple comparisons. The significance level was set at $P = 0.05$. All data were plotted as mean $\pm$ standard error.

For Study IV, the differences between all groups in the histologic analyses were evaluated using non-parametrical Kruskal-Wallis test (SPSS Statistics v. 22, IBM Corp., USA). Mann-Whitney U test was used to compare the histomorphometrical values within same group between 2 and 6 weeks. The significance level was set at $P = 0.05$. All data were plotted as mean $\pm$ standard deviation.

RESULTS

Systematic review

Literature search: The electronic and hand searches yielded 352 titles, of which 106 were screened after evaluation of titles and abstracts. A number of 45 articles were excluded either because they did not meet the inclusion criteria (35 papers), or because they were cited in more than one database (10 papers). Thus, 61 papers were included in the review (Figure 7) ^{120, 121, 125, 130, 131, 136, 140, 141, 152, 157, 183, 253, 254, 258, 259, 269-314}. Rats, dogs, and rabbits were the most commonly used animals in the studies (34%, 33%, and 23% of the studies, respectively).

Main methods for local drug delivery

Two main methods were utilized for local drug/chemical compound delivery around implants. The most commonly used method was by incorporating or immobilizing chemical agents into implant surfaces (n=44), while the second method involved local applications of chemical agents to the implants site either directly or using carrier materials (n=17).

Chemical agents were incorporated, coated, or immobilized on implant surfaces

Different techniques were reported for implant coating or immobilizing with chemical agents such as: by pipetting ²⁸¹, by immersion ³¹⁰, by plasma-spray process ²⁷⁰, or by electrophoretic deposition technique ²⁶⁹. In this review, twenty-two studies reported mean BIC values with standard deviation (SD), and the

statistical analysis showed significantly higher BIC values for coated in comparison to uncoated implants ($p=0.02$).

Chemical agents were locally delivered at the implant site

The carrier materials used for local drug release at the implant site were synthesized in different forms such as: injectable gels ^{253, 285}, microsphere hydrogels ^{259, 295}, or using collagen sponge ²⁷¹. Twelve studies reported mean BIC values with SD used for the meta-analysis. The analysis showed significantly higher BIC for implants with local drug and chemical compounds delivery at implant sites compared to implants with no drug ($p<0.0001$).

Figure 7. Study screening process.

Type of Drugs/Chemical Compounds

Three groups of drugs and chemical compounds were evaluated more frequently: growth factors ($n=30$), bisphosphonates ($n=16$), and calcium phosphate ($n=14$). In some studies, more than one type of chemicals were used. Furthermore, there were enough studies to

do meta-analyses for these three types of chemical compounds. Implants coated with calcium phosphate (CaP) were associated with significantly higher BIC compared to uncoated implants ($p=0.0001$). A significantly higher BIC was observed with local application of bone morphogenetic protein (BMP) on implant surfaces ($p=0.02$). For bisphosphonates, the meta-analysis showed no significant difference when using these drugs on implant surfaces compared to control implants ($p=0.15$).

Characterization of materials

Differential scanning calorimetry (DSC)

In Study II, DSC analysis was used to determine the lower critical solution temperature (LCST). Figure 8 exhibits the DSC thermograms of the PNIPAAm and PNIPAAm-AAm polymers. For the conventional PNIPAAm, the LCST was determined at approximately 34°C, which correspond with what have been reported in most other studies. PNIPAAm-AAm having 5% and 10% substitution showed a LCST at approximately 40°C and 42°C, respectively.

Figure 8. DSC scans of PNIPAAm and PNIPAAm-AAm polymers (at a heating rate of 1 °C /min). All data is presented as a mean value (n=3).

Scanning electron microscopy (SEM)

In Study II, scanning electron microscopy (SEM) was used to visualize the PNIPAAm coating supported by a mesoporous titania film. At high magnification, the top view images showed a well-defined porous structure with a pore size determined at 6 nm, covering the whole titanium surface (Figure 9). With the cross section view, the SEM showed a uniform mesoporous film around 300 nm in thickness (Figure 10), while the polymer layer on top of the mesoporous film was around 200 nm in thickness (Figure 11). Meanwhile, a top view image for the polymer film with the incorporated GNRs showed a homogeneous distribution of GNRs on the first polymer layer, which is crucial to preserve the strong absorption of NIR light (Figure 12).

Figure 9. A top view SEM image for the mesoporous titania film.

Figure 10. A cross section SEM image for the mesoporous titania film.

Figure 11. A cross section SEM image for the mesoporous titania film and PNIPAAm layer.

Figure 12. A top view of the GNRs on the PNIPAAm. The inset shows the rods at higher magnification.

Figure 13 shows the SEM images for titanium implant screws coated with PNIPAAm before and after insertion into the mandible bone of a dead moose. There were no observed differences between the coated screws before and after the removal from the prepared site, which indicate no detachment of the coating.

Figure 13. SEM images showing: (A and B) uncoated implant screws, (C and D) screws coated with PNIPAAm, and (E and F) screws coated with PNIPAAm were inserted then removed from bone.

In Study III, the PLGA microspheres morphology was examined under high-magnification using SEM. Different sizes of microspheres were observed with an average size around 26.4 μm (Figure 14).

Figure 14. SEM image showing clarithromycin encapsulated PLGA.

In Study IV, the SEM evaluation for P123 and P123-PPG mesoporous films revealed a well-ordered porous structure formed on titanium discs (Figures 15 and 16). The calculated pore size was 6 nm for the film synthesized with P123, and 7.2 nm for the film synthesized by adding PPG to P123.

Figure 15. SEM micrograph of mesoporous TiO₂ thin film formed with P123 template.

Figure 16. SEM micrograph of mesoporous TiO₂ thin film formed with P123+PPG template.

Quartz crystal microbalance with dissipation monitoring (QCM-D)

The shrinkage behavior of the polymer layer upon NIR irradiation

In Study II, the QCM-D analyses revealed a rapid increase in frequency for the polymer-coated crystals with or without GNRs from the moment the NIR irradiation started (Figure 17). The frequency of the polymer-coated crystals incorporated with GNRs increased 500 Hz, while the film without GNRs increased only 25 Hz. When the NIR was stopped, the frequency decreased gradually to return to the same value as before the irradiation, indicating that the properties of the polymer layer are reversible.

Figure 17. QCM-D recordings showing frequency changes of the sensor crystal coated with PNIPAAm (with or without GNRs) upon irradiation with NIR. The asterisks indicate the time when the NIR was turned on and off.

The accessible pore volume: In Study II, the pore volume of PNIPAAm was calculated by exchanging water (H₂O) with deuterium oxide (D₂O) (Figure 18). The water content occupied within the 100-nm polymer coating was approximately 13.7%.

Figure 18. QCM-D results showing frequency and dissipation after (H₂O/D₂O) exchange on coated and uncoated sensors with PNIPAAm. The asterisks indicate the time when (H₂O/D₂O) was exchanged.

Strontium ranelate absorption and release rate

In Study IV, the QCM-D was used to monitor strontium ranelate absorption and release from two modified mesoporous surfaces with 6 and 7.2 nm pore sizes (Figure 19). Significantly higher absorption and release of strontium was seen from P123-PPG with 7.2 nm pore size compared to P123 (6 nm pore size). Both groups showed similar release kinetics and sustained release of strontium ranelate after solution exchanged with milli-Q water.

Figure 19. QCM-D results showing Sr-ranelate absorption and release from MT films. The asterisk indicates the time when Sr-ranelate solution was exchanged with flow of Milli-Q H₂O.

UV/VIS spectroscopy

GNRs absorption of NIR

In Study II, the dispersion of the PEG-modified GNRs was recorded using UV/VIS spectroscopy. The PEG-modified GNRs showed an absorbance peak at 850 nm that corresponds to the peak value found for GNRs stabilized with CTAB (Figure 20). Thus, the addition of PEG did not cause the GNRs to aggregate. The NIR light source used was an LED lamp (ThorLabs LDC200C) with a maximum intensity of 1 W/cm² at 850 nm.

Figure 20. UV/VIS spectra for: bare GNRs, GNRs modified with PEG, and the intensity measures of the LED lamp at wavelength spectrum.

Phenol release behavior

Phenol was used as a model drug to evaluate the drug release behavior of the PNIPAAm-coated surfaces upon irradiation with NIR by the use of UV/VIS spectroscopy. The standard calibration curve of the absorbance of phenol concentration at 269 nm is shown in Figure 21. Before irradiation with NIR, stable levels of phenol concentration were observed around all coated surfaces. Upon irradiation, immediate and significant increase in phenol release was observed from PNIPAAm with GNRs (Figure 22). The phenol release continued even after the irradiation was ended. Meanwhile, the polymer in the control group (no GNRs) showed a slight release of phenol with NIR irradiation.

Figure 21. Standard calibration curve of the absorbance of phenol concentration at 269 nm using UV/VIS spectroscopy.

Figure 22. Changes in the phenol concentration upon irradiating the coated surfaces with NIR.

Release profile of Clarithromycin

The *in vitro* release profile of clarithromycin from the microspheres presented sustained release for 4 weeks (Figure 23). The microspheres released around 30% of clarithromycin in the first 3 days, and around 50% during the first week.

Figure 23. *In vitro* release profile of clarithromycin-loaded PLGA microsphere.

Bacterial test

The antimicrobial effect of GNRs was evaluated on GNRs-incorporated PNIPAAm surfaces (without antibiotics) before and after NIR irradiation (Figures 24 and 25). Before NIR irradiation, a high number of adhered and live (green) bacteria was observed on the PNIPAAm surfaces. Meanwhile, after NIR irradiation, the majority of these bacteria was recognized as dead (red), in accordance with previous observations ³¹⁵.

Figure 24. Fluorescent image for cells present on the GNRs-incorporated PNIPAAm before irradiation with NIR.

Figure 25. Fluorescent image for cells present on the GNRs-incorporated PNIPAAm after irradiation with NIR.

Furthermore, the antibacterial behavior of the coating with the presence of the antibiotic was evaluated by studying the growth inhibition zone around coated glass surfaces in response to NIR. Before irradiation with NIR, all groups demonstrated a dense *Staphylococcus epidermidis* population readily surrounding the samples. After irradiation for 10 minutes, no clear change related to the bacterial growth was seen in groups 1 and 2 (Figure 26 and 27). The samples from group 3 (GNRs-incorporated polymer and loaded with vancomycin) demonstrated a clear inhibition zone around the coated glasses (Figure 28).

Figure 26. Representative images showing *Staphylococcus epidermidis* growth around the samples from group 1 (Polymer loaded with antibiotic without GNRs) before NIR irradiation (left) and after irradiation (right).

Figure 27. Representative images showing *Staphylococcus epidermidis* growth around the samples from group 2 (GNRs-incorporated Polymer without antibiotic) before NIR irradiation (left) and after irradiation (right).

Figure 28. Representative images showing *Staphylococcus epidermidis* growth around the samples from group 3 (GNRs-incorporated Polymer with antibiotic) before NIR irradiation (left) and after irradiation (right).

In vivo results

Micro-computed tomography

In Study III, three-dimensional micro-computed tomography (μ CT) images were constructed to evaluate bone formation within bone defects (Figure 29). The results obtained from μ CT evaluation showed statistically significant higher amount of bone formation in the defects treated with β -TCP combined with clarithromycin-loaded microspheres compared to the sham group (Figure 30). The significant difference was seen during all healing times, ($p= 0.00$, 0.014 , and 0.002 in 2, 4, and 12 weeks, respectively). Statistically significant difference was evident between β -TCP with clarithromycin and sham after 2 weeks ($p= 0.009$).

Figure 29. 3D reconstructed images using μ CT after 2,4,and 12 weeks for: (1) sham, (2) β -TCP, (3) β -TCP and clarithromycin, (4) β -TCP and PLGA with clarithromycin.

Figure 30. CT scan: New bone formation % in the bone defects.

Histomorphometry

In Study III, the qualitative evaluation for the histological sections revealed bone formation localized mostly at the periphery of the defects among the four groups after 2 weeks of healing (Figure 31). After 4 weeks, all groups continued to demonstrate more bone with some bone areas approaching the center regions of the defects. There were no significant differences observed between the defects augmented with β -TCP compared with sham at 2 and 4 weeks of healing time. However after 12 weeks, β -TCP mixed with microspheres loaded with clarithromycin presented statistically significant higher amount of newly formed bone compared to the sham ($p= 0.002$). The quantitative measurements are summarized in Figure 32.

Figure 31. Descriptive histological micrographs after 2, 4, and 12 weeks of healing for all groups; (a) sham (group 1), (b) β -TCP (group 2), (c) β -TCP & clarithromycin (group 3), (d) β -TCP & PLGA with clarithromycin (group 4). Scale bars represent 500 μ m.

Figure 32. Bone area % within the defect.

In Study IV, the histomorphometric analyses for BIC, BA, and new-BA are summarized in Table 1 and Figures 33-40. After 2 and 6 weeks, higher BIC and BA were seen with implants incorporated with Sr-ranelate compared to implant in control groups, but these differences were not significant. The *p*-value for BIC was 0.430 at 2 weeks and 0.172 at 6 weeks, while the *p*-value for BA was 0.503 at 2 weeks and 0.088 at 6 weeks. The histomorphometrical analyses within same group after 2 and 6 weeks are shown in Figure 36. The mean BIC and BA values within the same group showed significant increase among all groups between 2 and 6 weeks.

The qualitative evaluation for the histological sections revealed layers of newly formed bone lining the implant threads characterized many of the implants incorporated with Sr-ranelate (Figure 40).

Table 1. Summary of the histomorphometric measurements (Study IV).

Group	BIC % (SD)		BA % (SD)		New-BA % (SD)	
	2 weeks	6 weeks	2 weeks	6 weeks	2 weeks	6 weeks
Control (6 nm)	52% (11)	71% (8)	42% (8)	51% (10)	34% (6)	34% (13)
Control (7.2 nm)	51% (12)	66% (11)	42% (8)	52% (12)	35% (10)	39% (11)
Sr (6 nm)	58% (10)	76% (9)	47% (7)	60% (8)	34% (8)	38% (6)
Sr (7.2 nm)	59% (8)	76% (9)	45% (6)	59% (7)	35% (5)	33% (7)

Figure 33. Bone-implant contact percentage along threads. *P* value =0.43 at 2 weeks and 0.172 at 6 weeks.

Figure 34. Bone area percentage between threads. *P* value =0.503 at 2 weeks and 0.088 at 6 weeks.

Figure 35. Percentage of new bone area between the threads. *P* value =0.78 at 2 weeks and 0.464 at 6 weeks.

Figure 36. Histomorphometrical values; BIC%(A), BA%(B), and new-BA%(C), within the same group between 2 and 6 weeks. The significance level was set at $P = 0.05$.

Figure 37. Histological micrographs obtained after 2 weeks of healing for implants coated with mesoporous films: (a) 6 nm, (b) 7.2 nm, (c) 6 nm loaded with Sr-ranelate, and (d) 7.2 nm loaded with Sr-ranelate. Scale bar is 200 μ m.

Figure 38. Histological micrographs obtained after 6 weeks of healing for implants coated with mesoporous films: (a) 6 nm, (b) 7.2 nm, (c) 6 nm loaded with Sr-ranelate, and (d) 7.2 nm loaded with Sr-ranelate. Scale bar is 200 μ m.

Figure 39. Histological observation at high magnification for early bone formation around implants coated with mesoporous TiO_2 after 2 weeks of healing. New bone starts to fill the areas between cortical bone and implant. Cortical bone (CB) is visualized in pale red while the new bone (NB) is visualized in purple.

Figure 40. Histological observations at high magnification for formed bone along the threads for the screw coated with mesoporous film (P123) loaded with Sr-ranelate after 6 weeks of healing time. Large number of osteoblasts can be seen over the formed bone layer to help build new bone. Cortical bone (CB) is visualized in pale red while the new bone (NB) is visualized in purple.

DISCUSSION

Systematic review

Systematic reviews are scientific investigations that involve an extensive and reproducible search of different sources to answer pre-planned research question. The search strategy for a systematic review should start with a clear research question to help collecting all the relevant studies (as much as possible) to answer the question. To help identifying all relevant studies, it has been suggested for any planned search strategy to include a systematic search through multiple databases in addition to hand searching³¹⁶. This approach is believed to reduce bias and help reaching a reliable estimates of effects³¹⁷. In some situations, the systematic review can be combined with statistical evaluation using meta-analysis to examine a specific research question³¹⁸. The meta-analysis is a review that involves statistical methods used to evaluate the numeric data from the primary studies. It can help produce a summary of the numeric results of several studies and identify the methodological weaknesses that might greatly affect the results³¹⁹.

For the systematic review included in this thesis (Study I), the aim was to investigate the methods most commonly used for local drug and chemical compound delivery to implant sites and assess their influence on bone response.

The search for the relevant studies was: electronic search through databases, hand searching, the reference list of the studies, and the relevant reviews on the subject. The used databases (PubMed, SCOPUS, and Embase) are major biomedical databases with a strong

focus on medicine and health related subjects. After data collection, a total of 61 publications met the inclusion criteria and were included in the review^{120, 121, 125, 130, 131, 136, 140, 141, 152, 157, 183, 253, 254, 258, 259, 269-314}. CaPs, BPs, and BMPs were the most commonly used drugs/chemical compounds in implant experiments as been shown with this review.

CaP: Interestingly, CaP demonstrates chemical similarity to the mineral component in natural bone ³²⁰. Some reports revealed enhancement of bone cells adhesion/activation on the surface of implants when coated with CaP ³²¹. From the studies included in this review, different results were seen with local application of CaP to implant surface. This may be attributed to particular features of the CaP coating process, different CaP phases, different animal models, implant patterns/surfaces, and biomimetic processes ¹³⁰. The meta-analysis revealed that implants coated with CaP showed a statistically higher BIC compared to implants without CaP. The higher BIC may be related to the fact that implants coated with CaP offer a physicochemical matrix proper for the deposition of new bone by osteoblasts, which could explain the increased BIC ^{322, 323}.

BPs: BPs belong to a group of drugs known to inhibit bone resorption and renewal that are mediated by osteoclasts. Therefore, BPs may increase mineralization under normal function and allow the increase in bone mineral density ³²⁴. Some investigations showed that BPs could increase the osteogenic differentiation of the mesenchymal stem cells ³²⁵. Zoledronate and alendronate are examples of BPs drugs commonly used around implants ^{272, 311}. The statistical analysis for the effects of BPs revealed no significant difference between implants/implant sites presenting BPs compared to control implants/implant sites without BPs. One of the suggested explanations for this finding is that the prevention of osteoclast-mediated bone resorption and renewal influenced by BPs, could lead consequently to retaining of old bone. Some reports claimed that osteoclast-osteoblast interaction increased with BP and could slowdown bone remodeling. Aspenberg et al.³²⁶ suggested that BPs bind to bone mineral and are taken up by osteoclasts

during bone resorption. Nevertheless, other studies showed improved implant fixation with local release of BPs ^{327, 328}. BPs administration together with implant treatment was suggested for many patients suffering from low bone density. However, there are some concerns regarding the delivery of BPs using the conventional methods. Some studies showed that systemic use of BPs might cause osteonecrosis in the jaw. However, many other studies have not shown this effect ³²⁹. Local administration of BPs can be seen as a good alternative to limit any possible side effects that may occur with conventional methods.

BMPs: Many reports showed that BMPs might enhance differentiation of immature mesenchymal-derived cells ^{330, 331}. Thus, it was examined with implants at different clinical settings to assist in bone formation ^{125, 332-334}. Local applications of BMPs to implant surfaces were analysed by different methods ³³⁵. Some of used techniques involve using carrier materials to deliver BMP to implant site^{336, 337}, while others are simply based on coating the implant surface with BMP ²⁸⁶.

Coating of BMP on implant surfaces can allow the release of the BMP directly into bone-implant interface. However some limitations appeared with the delivery of BMP using such techniques. Some investigations showed unstable bonding between BMP-2 and implant surfaces when BMP-2 was coated to the surface ^{338, 339}. This could cause BMP-2 to be driven away from the target area at early stage and consequently losing their therapeutic effects. One approach to overcome this limitation is by bonding BMP to a Ti implant surface although this may cause protein denaturation during the cross-linking process ³⁴⁰. Slow BMP release from implant surfaces has been examined in many studies using biomimetic implant coatings ^{297, 341}. Alterations in osteoclasts activity with early bone maturation were observed using this technique ²⁹⁷.

The local delivery of BMP to implant site using carrier materials was investigated numerously as an approach to control the BMP release ^{275, 335, 339, 342, 343}. Various carrier materials were employed for this purpose, including: titanium sponges ³⁴⁴, collagen sponges

²⁷⁹, atelopeptide type-I collagen ²⁷³, hyaluronic acid hydrogel ¹⁸³, polylactic acid membranes ³⁴⁵, and by bioactive carriers ^{184, 339}.

The meta-analysis for local release of BMP showed that it might lead to an improvement in BIC. However, this improvement was not seen in all studies. This could be explained by the variation between the methods of application, different carriers, time of application, and animal models.

Not surprisingly, concentration of BMP can play an important role in implant osseointegration. High concentrations of BMP showed superior results in several experiments when compared to low concentrations ^{125, 286, 288, 346}. However, the outcome was also associated with some side effects such as inflammation and abnormal bone formation ^{347, 348}. It is important to pay attention to BMP concentration since this agent is believed to demonstrate dose-dependent properties with regard to osteoclast activity and bone remodeling^{349, 350}.

Different animals and time periods

Small animals like rodents are employed extensively in implant research. Our findings support this fact since rats and rabbits were the most commonly used animals among the included studies. The great differences in bone biology in these animals compared to larger animals like dogs or pigs might also reveal differences in bone response, and therapy affecting the obtained results. This was seen in the meta-analysis when BMPs were added to implants. We observed for instance superior results in large animals compared to rodents. However, it is difficult to clearly suggest which animal or time point that would be the optimal one since there were only few studies for each animals/time periods. In addition, some studies do not have the statistical power to rule out the possibility of a difference between groups. Furthermore, comparisons between studies have shortcomings due to differences in protocol and technology ¹³⁰.

In vitro study

The unique volume change behavior that was demonstrated by PNIPAAm polymer in response to temperature has attracted

attention to be utilized in drug delivery applications ^{202, 351-353}. Investigations made on PNIPAAm in other applications in soft tissue and bone regeneration reported good biocompatibility for this material ^{354, 355}. It is very essential when using PNIPAAm on implant surfaces not to interfere negatively with the osseointegration process.

GNRs-incorporated PNIPAAm, synthesis and characteristics

The PNIPAAm polymers were synthesized with different ratios of acrylamide to obtain different LCSTs. Co-polymerizing PNIPAAm with acrylamide was reported to have no effect on PNIPAAm thermal sensitivity ³⁵⁶. We observed that LCST for PNIPAAm synthesized with 10% of acrylamide (weight ratio) is around 42°C. Phase transition at this temperature could be suitable for any future clinical usage since the polymer phase transition is a few degrees above normal body temperature, thus uncontrolled drug release from the polymer can be avoided. Meanwhile, higher temperature for phase transition could have risks to the living tissues since some reports showed that cortical bone necrosis can be induced at 47°C ³⁵⁷. Thus, the later analyses in this study were based on using PNIPAAm-AAm 10% only.

In addition, we supported the polymer layer with a mesoporous titania film. The SEM images revealed a uniform mesoporous titania film having a highly porous structure. Adding the mesoporous titania film in the drug delivery system can help increasing drug-loading capacity¹⁵⁴. The polymer was coated on Ti screws and examined in a lab test by inserting and removing the screws from prepared implant site. No detachments of the polymer coatings were observed, which suggests good mechanical stability.

In this study, we designed GNRs-incorporated PNIPAAm as thin coating while the NIR was used for the photo-thermal conversion to obtain the necessary increase in temperature to induce polymer phase transition. This was suggested for local and controlled drug release into the bone-implant interface. The increase in temperature caused by GNRs absorption of NIR is known to be dependent on GNR concentration ³⁵⁸. During the spin coating technique, which was used to form a thin polymer layer, the GNRs were spin coated

between two layers of polymer with the aim to decrease the direct contact between tissue and GNRs, and to maximize the thermal effects centrally inside the polymer layer. SEM images showed good distribution of GNRs on the polymer surface. In addition, incorporation of GNRs into PNIPAAm has been reported not to cause significant disturbances to the phase transition behavior of the polymer ²²⁴.

Irradiation with NIR light was reported to be harmless to the body tissue since wavelengths in the NIR region can penetrate deep in the body with very low absorption by water and hemoglobin ²²³. NIR has been applied for therapy in some portions of the brain at 3 centimeters depth through the skin and skull bone ³⁵⁹. The osseointegrated dental implant is expected to be surrounded by a few millimeters of alveolar bone under normal conditions ³⁶⁰. Therefore, the NIR is suggested to reach the coating with sufficient intensity needed to induce the photo-thermal conversion provided by GNRs.

Formation of the thin polymer layer and mesoporous film was achieved using the spin-coating technique. In the spin-coating technique, changing the concentration of the polymer solution or the speed of spin coater will allow the formation of different coating thicknesses.

The shrinkage behaviour of PNIPAAm (incorporated with GNRs) in response to NIR was examined in this study using QCM-D. Our analyses revealed a dramatic increase in the frequency after irradiation with NIR. Different factors can influence the frequency shift in a viscoelastic polymer including the layer thickness and density³⁶¹. In general, the increase in frequency is interpreted as a decrease in the adsorbed mass on the crystal surface. Therefore, the high increase in frequency in the QCM-D results could indicate absorption of NIR by the incorporated GNRs, resulting in shrinkage and loss of mass of the PNIPAAm layer. Meanwhile, a slight dehydration of the control group (PNIPAAm without GNRs) could be an explanation for the small increase in frequency. The increase was probably caused by NIR absorption by the crystals themselves.

PNIPAAm for drug delivery: We examined the PNIPAAm layer loading capacity using QCM-D by measuring the water uptake and release from the PNIPAAm coated crystals. The water content within the 100-nm polymer coating was found to be around 13.7%, which can be seen as low loading capacity. Thus, supporting the polymer layer with the mesoporous film might be an advantage since it will increase the loading capacity significantly.

The drug release test was performed by UV/VIS spectroscopy using phenol as drug model since phenol is characterized by low water solubility and high detectability ³⁶². The abrupt release of phenol seen with the GNRs-incorporated PNIPAAm was suggested a result of a physical collapse of the polymer film upon irradiation. The continued phenol release after the irradiation was stopped which may indicate that the drug release from the polymer coating cannot be totally halted, but only initiated by the use of NIR ³⁵¹. Meanwhile, the slight increase in the phenol release seen in the PNIPAAm layer without GNRs could be explained by a mild absorption of NIR by the polymer layer, which is somehow similar to the findings observed during evaluation of shrinkage behavior done by QCM-D measurements.

Bacterial test: To test the efficacy of this drug delivery system, *Staphylococcus epidermidis* was used to assess the bacterial growth around the PNIPAAm-coated surfaces loaded with vancomycin. Staphylococci bacteria are considered one of the frequently isolated pathogens associated with implanted medical devices ^{363, 364}.

The observations from the bacterial test suggest that the PNIPAAm-coated surfaces were able to maintain the antibiotic before irradiation. However, irradiation with NIR on GNRs-incorporated polymer loaded with antibiotic was associated with the formation of inhibition zones. Since the inhibition zones were observed only with coating loaded with GNRs and antibiotics, it is possible to state that the inhibition zones could be caused by the drug release after polymer shrinkage. These findings show that the polymer coating (as a carrier) may not affect or change the drug properties. The local antibiotic release around implants may be

considered a good alternative for other treatment modalities used for the treatment of peri-implant diseases ³⁶⁵.

For the drug delivery system described here, the drug release from the polymer coating could be initiated upon NIR irradiation. This release behavior may be useful in some cases of implant-associated infections where local drug release is needed at a later stage after implant insertion. Consideration of the chemical nature of the drug should be in mind in future studies when PNIPAAm is chosen as a drug delivery system.

***In vivo* study (Clarithromycin & PLGA)**

In this study, a drug delivery system based on poly (lactic-co-glycolic) acid (PLGA) incorporated in bone substitute (β -TCP) was used to investigate the clarithromycin release profile and whether the release would effect bone regeneration in bone defects.

The drug release analysis using UV spectrophotometer revealed sustained clarithromycin release from PLGA microspheres extended to 4 weeks. The release profile from MSPs showed the release of around 50% of clarithromycin during the first week. This was followed by slower and sustained release of the drug resulting in around 100% cumulative release for 3 weeks.

The drug release from PLGA microspheres was suggested to occur by diffusion of the drug from the microspheres. The drug release profile revealed an initial burst in the drug release pattern observed in the first week. The release progresses in a slow release after the first week as the degradation process of MSPs continues ³⁶⁶. In this experiment, the degradation process that PLGA demonstrated was assumed as the major factor in the sustain release of clarithromycin. Different reports suggested that the release mechanism from PLGA is influenced by different factors such as drug concentration and drug type ³⁶⁶⁻³⁶⁸.

PLGA is commonly utilized for encapsulation and release of wide range of drugs and chemical compounds ³⁶⁹⁻³⁷¹. Besides, the high biocompatibility and favourable biodegradable behaviour of PLGA attracts attention for usage in bone scaffold applications. However, the relatively low mechanical strength of PLGA compared to cancellous bone was seen as a limitation. Thus, incorporating PLGA

in bone substitute materials was suggested to improve its mechanical properties ³⁷². In a study done by Naito et al.,¹⁹² PLGA microspheres were loaded with simvastatin and incorporated into calcium aluminate cement in rabbit calvaria. The histologic analysis revealed that the microspheres containing simvastatin significantly enhanced bone formation compared to defects in the control group.

In the current study, β -TCP was used as bone substitutes material with or without the addition of PLGA. β -TCP is a biocompatible material with osteoconductive properties and has been investigated substantially in bone regeneration studies ^{373, 374}.

The evaluation of bone formation in the defects was done using histologic sections and μ CT images. The differences in bone formation measures were not significant when the bone formation was evaluated using histologic sections. The μ CT images allowed visualizing the entire biologic phenomenon when evaluating bone formation. Thus, μ CT images may be more descriptive (compared to the histological images) when evaluating bone formation in the defect area. The evaluation performed using histological images present only a single cut in the middle of the defect.

Improvement in bone formation was observed during the addition of clarithromycin within the bone substitutes. This improvement was seen after 4 weeks even when the PLGA microspheres was not used with the bone substitute. Meanwhile, clarithromycin loaded in MSPs succeeded to show continued bone formation even at 12 weeks.

The findings from the present experiment showed that the sustained release profile of clarithromycin from MSPs was associated with the highest regeneration of new bone at extended healing periods. The sustained release behaviour of MSPs can motivate for more experiments using numerous drugs.

Some reports showed that clarithromycin helped to restrict biofilm formation in different types of aerobic and anaerobic bacteria ³⁷⁵. Additionally, clarithromycin has been reported to exhibit anti-inflammatory effects ^{376, 377}. These features somehow influence bone regeneration since excessive inflammation and infection leads to failure of the bone augmentation.

The favourable degradation process of bone substitutes (β -TCP) and PLGA may indicate an important role in enhancing bone formation ^{378, 379}.

The degradation process of PLGA occurs by the hydrolysis of its ester linkages in the water environment ³⁸⁰. This degradation process has been reported to produce acidic by-products that accumulate in the space created after microspheres degradation ¹⁶⁵. In this study, no biological side effects or healing problems were observed with the groups containing PLGA in the bone substitutes. Thus, our findings correspond with other reports that have suggested the positive effect of PLGA degradation behavior on bone formation.

In addition, degradation processes of β -TCP are believed to stimulate an osteogenic marker of osteoblastic cells developed from calcium ion release ³⁸¹. Meanwhile, other studies have demonstrated that β -TCP is degrading by macrophage-like giant cells without causing a cytotoxic response ^{382, 383}.

In a previous histologic investigation by Yoshida et al., ³⁸⁴ the encapsulated β -TCP was characterized by newly formed bone and cell-rich tissue, including giant cells. They suggested, after histological observations performed after 10 and 35 days, that degradation of β β -TCP and osteogenesis occurred at the same time. The bone formation has been reported to take place within bioresorbable biomaterials at the resorption site ³⁸⁵. Employing fast resorbing β -TCPs can support bone regeneration without interfering with the long-term health of bone at the site. Thus, filling of bone defects with such bone substitute materials with the addition of clarithromycin could be a suitable approach for the filling of bone defects. Other factors such as density and stability play an important role in bone formation for the used bone substitute ³⁸⁶.

In vivo study (Sr-ranelate)

Study IV investigated the release of Sr-ranelate from an implant surface coated with mesoporous TiO₂. Some previous reports have indicated the positive influences of strontium administration on bone formation in implant sites ^{387, 388}. However, the use of

conventional methods for strontium application showed some incidences of side effects ³⁸⁹. Thus, local application of strontium into the bone-implant interface was seen as good alternative to minimize any possible side effects while allowing drug release at a minimal but efficient concentration.

A common method for drug release around implant surface is by coating or incorporating the drug agent into implant surfaces. For strontium, different techniques were investigated such as Sr-substituted HA coatings ^{390, 391}, plasma spraying ³⁹², and sol-gel technique ³⁹³. Most of the used techniques involve the deposition of Sr on implant surfaces that revealed modifications in surface morphology ^{390, 391}. Offermanns et al.³⁹⁴ investigated functionalized implant surfaces with nano-topography for local administration of Sr in ovariectomized rats. The implants were sputtered with nanostructured Ti-Sr-O coatings and revealed sustained release of Sr and accelerated osseointegration.

The long-stand effects during sustained release of strontium could be beneficial in terms of stimulating bone formation. Long-lasting and controllable strontium release was obtained using titanium nanotubes, which was associated with improved mesenchymal stem cell proliferation and osteoblastic differentiation ³⁹⁵.

In the current study, the implant screws were coated with mesoporous TiO₂ films to achieve sustained release of Sr-ranelate. The mesoporous TiO₂ was synthesized using different templates (P123 and P123-PPG) to demonstrate different pore sizes. This was confirmed by SEM images that showed uniform mesoporous films with 6 nm (for P123) and 7.2 nm (for P123-PPG) pore sizes. The addition of a swelling agent such as PPG at predetermined ratios allows the control of the mesoporous film pore size ^{262, 313}. This will enable the release of drugs agents with different molecular sizes. The mesoporous TiO₂ films were loaded with Sr-ranelate by immersion. The addition of Sr-ranelate is hypothesized to not cause significant change in the morphology of the films. Thus, all the examined surfaces in this study are assumed to show similar surface topography, which was obtained by the mesoporous TiO₂.

Therefore, the differences in bone response, if existing, may be a result of Sr-ranelate release.

Changing the surface characteristics of the mesoporous film may modify drug-loading capacity. The drug-loading capacity can also be correlated with the pore volume and surface area ^{266, 396}.

Cecchinato et al.³⁹⁷ investigated *in vitro* the absorption and release of magnesium from MT implant coatings with pore sizes similar to the ones used in this study. The authors found higher amount of magnesium absorbed into the MT coating with 7 nm (450 ng/cm²) compared to 6 nm (300 ng/cm²). Our QCM-D results demonstrated higher amount of immobilized Sr compared to what have been reported with magnesium. When the projected screw surface area, 0.2 cm², is taken into account this corresponds to 660 ng (7.2 nm) and 240 ng (6 nm) Sr-ranelate loading into the MT coatings per implant. If we then assume a diffusion distance of 1 mm, the distribution volume is at the order of 0.1 cm³. The resulting Sr-ranelate concentrations become 6.6 µg/ml (7.2 nm) and 2.4 µg/ml (6 nm). These Sr concentrations are within the optimized values of 0.21 and 21.07 µg/ml of Sr ranelate that has been shown to induce mesenchymal stem cells to differentiate into osteoblasts as determined in a previous *in vitro* study by Sila-Asna et al.³⁹⁸ Furthermore, the authors showed that high concentrations of up to 210.7 µg/ml might be associated with delayed effects on osteoblastic differentiation. The effect of strontium on bone have reported to be complex and dose-related ³⁹⁹.

In the current study, a rat animal model was selected to evaluate bone formation around implants with or without Sr-ranelate and was evaluated after two and six weeks. Although the rat bones have biological dissimilarities to human bones ⁴⁰⁰, they are common in implant research ¹²⁶. Some of the biological dissimilarities between rat and human bone is that rats have a shorter healing time and bone turnover period and lack a Haversian canal system ⁴⁰¹. However, the shorter healing time may facilitate the evaluation of osseointegrated implants at different healing stages ⁴⁰¹. The rats were examined at two weeks to investigate the early bone response. Meanwhile, the six weeks evaluation may allow observation of more completed osseointegration. Some studies showed comparable BIC

results after 6 and 12 weeks in a rat model ³⁹⁴. The miniature titanium screws were utilized in this experiment to accommodate the small bone size of the rats.

For the histomorphometric analyses, BIC, BA, and new-BA were used to study the quantity of osseointegration. These parameters represent a two-dimensional analysis utilized to observe bone formation along implant threads. The histomorphometrical analyses revealed no significant differences between the test and control implants. Although the beneficial effects of incorporating strontium into implant surfaces have been reported in numerous studies, the absence of significant differences between the control and test implants in this work could be related to the low amount of Sr-ranelate released compared with some other reports ^{127, 398}. In addition, the histological analysis evaluated a single section of the implant and did not capture the entire bone formation condition around the implants. The Sr-ranelate has been used frequently in the medical field mainly for the treatment of osteoporosis due to its anti-osteoporotic properties ^{389, 402}. Therefore, many of the animal experiments with strontium have tried osteoporotic animal models ¹²⁶. Thus, the effects of strontium on bone might be less evident compared when using animals with normal bone condition, like the animals used in this study. Meanwhile, small animals like rats were reported to have great healing capacity, which could make observation of different histological values not an easy task ⁴⁰³.

The comparable histological results seen in control and test implants could be related to the presence of the mesoporous films. The mesoporous films have been reported previously to stimulate cell attachment and differentiation. The mesoporous films can increase the surface area and facilitate implant integration with the bone. Interestingly, early bone apposition lining the implant threads was observed around implants containing Sr-ranelate. These findings could depend on the change in surface chemistry caused by Sr-ranelate, which may promote osteoblastic differentiation.

CONCLUSIONS

(Systematic review)

- Incorporating or immobilizing drugs/chemical compounds into the implant surface is considered the most common used method for local drug/chemical compound delivery around implants. A second common method for local drug delivery involves using carrier materials like gels or degradable polymers incorporated with chemical agents. Most of the studies assessing local drug/chemical compound release in implants evaluated the use of three groups of chemicals: BMPs, bisphosphonates, and calcium phosphates. Implants carrying these chemical compounds showed an overall improvement in bone implant contact when compared to control implants.

(In vitro)

- The new developed drug delivery system made of a thin layer of PNIPAAm co-polymer coating was incorporated with gold nanorods (GNRs) to support local heating of the polymer by photothermal conversion of NIR light. Co-polymerization of the PNIPAAm with 10% of acrylamide increased the LCST from 34 to 42 °C to better suit clinical

applications. Controlled release of phenol from the coating was obtained after irradiation with NIR light. Proof-of-concept of the drug-delivery system was demonstrated by releasing the antibiotic vancomycin to eradicate *Staphylococcus epidermidis* bacteria in culture.

- The biodegradable characteristic of PLGA microspheres successfully demonstrated an initial burst release of clarithromycin in the first week. After that, a slow release of clarithromycin was observed with a release duration lasting for more than 1 month.
- The mesoporous TiO₂ films with different pore sizes showed similar release kinetics of Sr-ranelate. The films allowed an initial fast release of Sr-ranelate followed by a sustained release.

(In Vivo)

- The *in vivo* findings revealed that incorporation of clarithromycin-loaded microspheres within beta-tricalcium phosphate (β -TCP) cement could stimulate bone formation in bone defects.
- After 2 and 6 weeks of healing, the *in vivo* experiment using rats could not confirm the positive effects of Sr-ranelate on implant incorporation in bone previously presented by other authors.

FUTURE PERSPECTIVES

- The evaluation of the *in vivo* studies revealed an overall improvement in bone contact with implants that were coated or incorporated with chemical compounds like BMPs, bisphosphonates, and calcium phosphates. A long term human clinical investigation is necessary to assess the effects of local release of these chemical compounds on bone formation.
- For GNRs-incorporated PNIPAAm, future works should be focusing on enhancing the drug release profile to demonstrate sustained drug release. Any potential *in vivo* and preclinical investigations need to assess the effects of implant coating with PNIPAAm on bone formation and whether the local drug release from PNIPAAm can assist in improving osseointegration.
- For the local drug release using PLGA microspheres, further investigations are recommended to improve the sustained drug release and to reduce the burst release, which could prolong drug availability in the healing site. In addition, further investigations are suggested to evaluate different drugs and scaffold materials to test their effects on bone formation.

- The effect of strontium release from mesoporous-coated implants was found not reinforcing the bone response in rats' tibia in our study. However, strontium still needs to be investigated with various drug amounts and duration, since only two concentration of strontium were tested at 2 and 6 weeks of follow up.

POPULÄRTVETENSKAPLIG SAMMANFATTNING

Introduktion

Implantatretinerade protetiska konstruktioner är idag ett vanligt och ofta prioriterat behandlingsalternativ för att ersätta förlorade tänder. Åtskilliga publikationer visar på mycket goda kliniska resultat inklusive mycket nöjda patienter. Emellertid finns det vissa grupper av patienter som visar fler misslyckade behandlingar än en normal population. Ett exempel på en sådan grupp kan vara diabetespatienter som kan ha en reducerad benläkningskapacitet vilket kan försämra inläkningen av ett implantat. En möjlig lösning för att förbättra lyckandefrekvensen för behandlingen av dessa patienter kan vara att täcka implantatet med en beläggning som lokalt administrerar benstimulerande läkemedel. På detta sätt kan läkemedlet frisättas långsamt i låg dos men ändå behålla terapeutisk effekt. Dessutom distribueras läkemedlet bara till det område där effekt önskas vilket kan minska potentiella bieffekter.

Material och Metod

I denna avhandling ingår fyra delarbeten (I-IV). Studie I är en litteraturstudie där dokumentation om olika metoder att belägga implantattytor med läkemedel kartlades. Vilka läkemedel som var vanliga för denna applikation undersöktes också. Dessutom gjordes en metaanalys för att undersöka vilka av metoderna och vilka läkemedel som visat på en förbättrad beninläkning av implantat.

I studie II, utvecklades ett nytt material för beläggning av implantattytor. Materialet är baserat på en värmekänslig polymer,

PNIPAAm, innehållande små guldpartiklar. Det nya beläggningsmaterialet kan laddas med läkemedel och frisättning av dessa initieras när ytan bestrålas med nära-infrarött ljus (NIR). Beläggningens egenskaper och förmåga till läkemedelsfrisättning undersökes i laboratoriet. Dessutom gjordes en utvärdering där bakterier användes för att undersöka antibiotika (Vancomycin) frisättning från PNIPAAm beläggningen efter strålning med NIR.

I studie III undersöktes frisättning av ett annat antibiotikum (Clarithromycin), från ett mikrosfäriskt PLGA polymert material. Denna polymer var blandad med ett benersättningsmaterial som användes för att täcka skapade bendefekter i kaninkranium. Mängden av ny benformation utvärderades med hjälp av histologi och skiktröntgen (μ CT) efter läktider om 2, 4 och 12 veckor.

I studie IV utvärderades frisättningen av strontium laddat i porösa titanoxidbeläggningar som deponerats på implantat. Effekten av strontium på benbildning undersöktes i en kaninmodell med läkningstider om 2 och 6 veckor.

Resultat och diskussion

Studie I visade att de mest vanliga sätten att administrera läkemedel kring implantatytan var att; (1) belägga implantatytan med läkemedel eller (2) att lokalt administrera läkemedlet till implantatområdet genom bärare. De flesta studier i litteraturgenomgången undersökte 3 huvudsakliga kemiska sammansättningar; Kalciumfosfat (CaP), bisfosfonater (BPs) och benmorfogenetiska proteiner (BMPs). Generellt påvisades förbättrad benformation med dessa substanser.

I studie II visade PNIPAAm polymeren goda fysikaliska och mekaniska egenskaper lämpliga för att kunna användas som bas för läkemedelsfrisättning från benförankrade implantat. Polymeren visade stark påverkan under bestrålning med NIR vilket kunde användas för att kontrollera frisättningen. Läkemedelsfrisättning kunde också konfirmeras i ett in vitro försök när bakterier som utsattes för frisättning av antibiotika dog.

I studie III visade mikrosfärisk PLGA långsam läkemedelsfrisättning som varade 1 månad. Mer ben bildades runt

de bedefekter som innehöll PLGA och antibiotika än utan dessa substanser efter 2, 4 och 12 veckors läktid.

I studie IV visade den porösa ytan möjlighet till kontrollerad läkemedelsfrisättning från implantat. Efter 2 och 6 veckor i *in vivo* försök kunde ingen skillnad i benformation påvisas mellan implantatytor som innehöll strontium och ytor som inte gjorde det.

Slutsats

Lokal läkemedelsadministration runt implantat visade sig kunna gynna benformation och förhindra infektion. Resultaten påverkades av typ av läkemedel och av administrationssätt. Implantat som belades med polymera material eller polymera material i form av mikrosfärer förbättrade kontrollen av läkemedelsfrisättningen. Läkemedels koncentration och frisättningsprofil påverkar avsevärt det slutliga resultatet.

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