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Surface bound bisphosphonate for implant fixation in bone

Rat experiments with a novel coating technique

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To my dear mother, father and grandmother!

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ABSTRACT

During the surgical preparation of bone, prior to insertion of an implant, bone will be traumatized which leads to local resorption. Consequently, early implant fixation might be reduced. Impaired early fixation, as evidenced by radiostereometry, has been associated with increased risk of late loosening. Bisphosphonates are known to inhibit bone resorption by osteoclasts and have shown to increase implant fixation when administered systemically or locally directly at the bone prior to implant insertion.

A method to bind bisphosphonates directly to the implant was developed. Stainless steel screws were coated with crosslinked fibrinogen, serving as an anchor for bisphosphonate attachment. The screws were inserted in the tibial metaphysis in rats and implant fixation was analyzed with pullout measurements. Bisphosphonate coated screws turned out to have 28 % higher pullout force at 2 weeks compared to control screws with the fibrinogen coating only. The next experiment was designed to measure at what stage in the healing process the strongest bisphosphonate effect was gained. Bisphosphonate coated screws were expected to reduce the resorption of the traumatized bone. However, no decreased fixation was found in the control group. Instead, the fixation increased with time, and so did the effect of the bisphosphonates. At 8 weeks, the pullout force was twice as high for screws with bisphosphonate compared to control screws. By histology at 8 weeks, a bone envelope was found around bisphosphonate coated screws but absent around control screws. Thus, the anti catabolic action of the bisphosphonate resulted in an increased amount of bone surrounding the bisphosphonate screws.

Titanium is generally considered to be better fixated in bone compared to stainless steel. The coating technique was found to be applicable on titanium as well, again with improved fixation.

A majority of fractures occur in osteoporotic bone. Despite the relatively low amount of bisphosphonates at the screws, the bisphosphonate coating improved implant fixation at 2 weeks also in rats made osteoporotic by ovariectomy.

In conclusion, bisphosphonates bound to titanium or stainless steel screws coated with fibrinogen increased fixation in bone, in rats. These results suggest that the bisphosphonate and fibrinogen coating might improve the fixation of screw shaped implants and possibly also arthroplasties, in humans.

LIST OF PAPERS

I. Wermelin K, Tengvall P, Aspenberg P. Surface-bound bisphosphonates enhance screw fixation in rats--increasing effect up to 8 weeks after insertion. *Acta Orthop* 2007;78(3):385-92.*

II. Wermelin K, Aspenberg P, Linderbäck P, Tengvall P. Bisphosphonate coating on titanium screws increases mechanical fixation in rat tibia after two weeks. *J Biomed Mater Res A* 86A:1, to be published in the July 2008 issue.*

III. Wermelin K, Suska F, Tengvall P, Thomsen P, Aspenberg P. Stainless steel screws coated with bisphosphonates gave stronger fixation and more surrounding bone. Histomorphometry in rats. *Bone* 2008;42(2):365-71.*

IV. Wermelin Karin, Tengvall Pentti, Aspenberg Per
A bisphosphonate coating improves the bony fixation of stainless steel screws in ovariectomized rats. Submitted for publication.

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INTRODUCTION

Implant fixation, clinical aspects

Why does fixation of implants in bone need to be improved?

With improved implants, the high risk of implant failure for younger patients might be reduced. Further, indications for orthopaedic or odontologic procedures might be widened.

The number of surgical procedures has been increased due to improved surgical techniques and implants. If the complications decrease further, it will be possible to treat even less severe cases with surgical procedures.

In orthopaedics, the incidence of total knee operations has increased greatly since 1975 and can be expected to continue to increase. However, the rate of arthroplasty revisions decreased considerably between 1979 to 1989 due to improved surgical techniques and better choice of implants. But still, in younger patients, the revision rate for total knee prosthesis, was approximately 25 % between 1988-1997, according to the Swedish knee register (99) and the revision rate for uncemented total hip prosthesis, mainly used on younger patients, was 27 % between 1992-2000, according to the Swedish hip register (79). This brings about an increased demand for longer implant survival.

One example of fracture fixation screws are external fixations pins, which are used in various clinical situations with diverging results (14, 54, 82, 93). Surgical procedures with external fixation pins are cheap and easily performed. It might thus be valuable to improve this technique further.

In odontology, the survival rate of dental fixtures in maxillae during a 15 year period has been reported to be 92 %, and the corresponding survival rate for mandibles was 99 %, in totally edentulous jaws (2). However, in order to achieve this high success rate the patients often have to wait between 3 to 6 months before the abutment and crown is inserted and function gained (21). A shortening of this healing period would improve patient comfort.

Why do orthopaedic prostheses and dental fixtures come loose?

In both orthopaedics and odontology, early micromotion has been suggested to contribute to implant loosening. Particle wear debris and overload are also considered important.

Integration of implants in the jaw bone and other bones in the body follows the same biological processes (20, 43). Early micromotion is considered to be a problem both in orthopaedics (66, 102) and odontology (41, 87) which often results in late loosening.

In odontology this problem can be diminished by protecting the dental fixtures from premature loading before the attachment of abutment and prosthesis. In contrast, an almost complete unloading is not possible in orthopaedics. However, the risk of infections is higher in odontology since even the slightest motion may direct bacteria from the oral cavity to the implant bone interface. This problem is attenuated through the separation of bone and implant from the oral cavity by coverage with mucosa. However, once dental prostheses are loaded they are exposed to high loads which may cause fracture of the surrounding bone or the implant (126). Loosening of orthopaedic joint replacements is often attributed to wear particle debris (released from the prosthesis generated by the articulation) that creates a chronic inflammation leading to bone resorption. However, even though chronic inflammations between the implant and bone is a problem in odontology, particle debris does not seem to exist in this field (4, 43).

In orthopaedics, revision of total hip and knee prostheses is mainly due to aseptic loosening or deep infection (9, 69, 79, 99) and is more frequent in younger patients (69, 79). External fixation pins are associated with infection and pin loosening (82, 93).

In odontology, early loosening of implants has been suggested to be mainly due to surgical trauma, lack of sufficient bone volume, deficient bone quality, infection and premature loading. A much higher incidence of implant failure has been reported in the maxilla than in the mandible. This has been suggested to be due to the smaller bone volume in maxillae, resulting in less mechanical resistance. (2) However, others have proposed that the high failure rates might be due to other reasons, such as severely resorbed jaws or poor bone quality, since with these cases excluded, the outcome is as successful as for mandibular jaws (5).

Implant fixation, biological aspects

What happens initially at the cellular level around the implant?

Where the implant is surrounded by compact bone the initial response is mainly resorption. Conversely, where the implant is surrounded by bone marrow, the initial response is dominated by new bone formation.

Implant screws in the marrow cavity, in animals

The following description is mainly based on light microscopic observations from two articles using a wound chamber model with titanium screws inserted in the mandible in dogs (1, 16) and an article using titanium screws inserted in the tibial condyle in rabbits (104).

The initial events around an implant in the marrow cavity are similar to wound healing. In the marrow cavity, a fibrin network with embedded erythrocytes and macrophages is formed next to the implant surface within a few hours after implant insertion. Three or four days later, the coagulum has been replaced by yearly granulation tissue and vascular structures have been formed around the implant.

Further, multinuclear cells are observed removing damaged bone fragments created during the surgical procedure. (1, 16) At 1 week, formation of new bone has started. In the studies on dogs, bone was formed around vascular units and at the implant surface, whereas in rabbits the formation of bone was more irregular and no formation of bone was seen at the surface. However, this dissimilarity could be an effect of the different implant models or animals used. At 2 weeks, the amount of newly formed bone has increased and in the study on dogs bone was seen in close proximity to the implant surface (1, 16, 104). Remodeling is noted after 4 or 8 to 12 weeks (1, 16).

Implant screws in the cortex, in animals

The following description is based on light microscopic observations mainly from an article using cylindrical titanium, fluorapatite and hydroxyapatite implants (38) but also from an article using threaded titanium implants (104) inserted in the tibial condyle in rabbits.

Three days after implant insertion the cut cortical bone is still in close contact with the implant. At some parts, however, there are gaps between the bone and the implant, and here erythrocytes embedded within a fibrin network are noted. At 1 week, osteoclastic bone resorption, represented by Howship's lacunae appear at the endosteal side of the cortex. Mesenchymal cells had begun to migrate into the gap and osteoclasts had begun to resorb at the implant to bone interface. These cells had apparently originated from the endosteal side. Woven trabecular bone is formed by apposition on the endosteal edges and protrudes towards the surface and is sometimes fused with new bone formed adjacent to the surface. (23, 104) At 2 weeks, formation of new bone has begun. The bone formation at the bone implant interface is characterized by osteoblasts on the original bone that produce osteoid that is subsequently mineralized. Mostly bone formation starts on the original bone directed towards the implant surface. However, for hydroxyapatite and fluorapatite coated surfaces, bone formation starting at the implant surface directed towards the original bone was frequently observed but more rarely seen for the uncoated titanium implants. Apart from that, up to this point, no major difference had been noted between the different implant surfaces.

Are there critical phases for fixation of implants in bone?

The attachment and retention of a fibrin network at the implant surface is considered to be important for direct apposition of bone at the implant surface. If resorption of the original bone prevails over the formation of new bone, the implant fixation might be at risk.

One of the initial events in bone healing around implants is the formation of blood clots at the implant surface. Several studies have shown increased bone to implant contact on a surface with a moderate degree of roughness compared to more smooth surfaces (23, 125). A rough surface may facilitate the attachment and improve the retention of a blood clot or function as a reservoir for bone promoting factors (23, 33, 34). Further, a rough surface poses a higher surface area than a smooth and may thus

contain a larger amount of adsorbed platelets that may secrete substances stimulating for proliferation and migration of other cell types. (34)

Bone traumatized during the insertion of an implant still has to serve as a mechanical support. However, as damaged bone is replaced by new bone, the fixation of the implant could become critical. A decrease in bone to implant contact was detected between 3 days and 4 weeks for cylindrical titanium implants inserted in cortical bone in rabbits (38). Another study, with threaded titanium implants, inserted in tibial metaphyses in rats, showed a decreased removal torque up to 4 weeks after insertion. However, within the same time interval, the pullout force increased continually. (21) This phenomenon has also been demonstrated in humans. Threaded titanium fixtures were inserted in the maxilla and exposed to direct loading. Stability measurements with resonance frequency analysis indicated that the screws were stable during the first week but lost stability during 3 months for the smooth implants and 2 months for more rough and oxidized implants. (49)

What constitutes the bone to implant interface?

Adjacent to titanium implants an amorphous layer has been observed suggested to serve as an anchor for collagen fibrils. This layer might have similarities with cement lines.

When a bone to implant interface is studied with light microscopy, no tissue between the bone and implant might be seen. This means that the implant is osseointegrated (4). However, in this case analysis with transmission electron microscopy could reveal the presence of a gap between the implant and the nearest collagen filaments in adjunction with the surrounding bone. (75) Several ultrastructural studies describe that the interface between bone and implants, made of commercially pure titanium (8, 75, 104, 105) or titanium subjected to anodic oxidation (23), is associated with an amorphous layer. However, the question whether these observations might be artifacts has been raised (74). It has been speculated that the amorphous layer might be formed by multinuclear giant cells at the surface in a similar way as when osteoclasts prepare for osteoblastic bone formation in a resorption lacuna. This could then explain why the amorphous layer has similar characteristics with cement lines. (104) The composition of cement lines is still under investigation but the calcium and phosphorus content was found to be lower compared to the surrounding bone (24, 130). The amorphous layer has been suggested to constitute a collagen free substance containing proteoglycans (20-40 nm) (75) or a collagen and mineral free ground substance (100-400 nm wide) (104, 105). Further, it has been suggested to function as an anchor for collagen filaments (4), embedded in the layer, and has also been observed around zirconium (7) as well as hydroxyapatite (119). With ultrastructural investigation of magnetron sputtered stainless steel (316L) a several hundred nanometer thick proteoglycan coat lacking collagen filaments was observed (6).

What influence does micromotion have on endosseous implant fixation?

A theory has been proposed that explains the tissue development around implants exposed to micromotion. Results indicate that hydroxyapatite is particularly advantageous for loose implants whereas bisphosphonates may improve implant fixation when the implant is exposed to smaller motions.

Implant micromotion depends on several factors such as; the surgical technique, available bone stock, status of the available bone, implant design, implant surface roughness, loading conditions, remodeling of host bone etc. Primarily, the biological reaction towards micromotion of endosseous implants will be discussed and secondly how micromotion can be prevented or halted.

Søballe et al. investigated the type of tissue formed, and how the tissue was altered with time, in a gap between the implant and the host bone as a response to implant micromotion. They found that initially, as the relative fluid velocity and shear tension at the surface of implant and host bone was high in the gap, fibroblast, and later chondrocyte, proliferation was promoted and the gap was subsequently filled with collagenous matrix. Consequently, due to the newly formed collagenous matrix the permeability was reduced causing a higher fluid pressure and a lower relative fluid velocity and shear tension, and further promoted chondrocyte proliferation. At a certain stage, when the implant was sufficiently stable, it was suggested that a motion-controlled situation would be replaced by a force-controlled situation. The mechanical environment would thus subsequently stimulate osteoblast proliferation. Alternatively, if sufficient stability would never have been reached the motion-controlled environment could have been preserved. Also in this case, the tissue would subsequently have developed a steady state but consisting of a fibrous or fibrocartilage tissue instead. (98) Van der Vis et al. showed that osteolysis occurred if fluid pressure was applied at the interface of host bone and implant which further demonstrate how cells are adapted to the mechanophysical environment. Peri-prosthetic loosening was suggested to be caused by implant micromotions producing fluid pressures at the interface of bone and prosthesis. (120)

For implants that had been continuously exposed to micromotion, the push-out strength for hydroxyapatite coated implants was approximately one and a half times higher compared to titanium alloy implants, exposed to equivalent micromotion. Further, if micromotion was ceased fixation of the titanium implant increased fourfold while the hydroxyapatite coated implant fixation only improved by 40 %. The authors suggested that the bone stimulating effects of hydroxyapatite coated implants are prolonged when exposed to micromotion (109). However, some studies confirm (29, 115) and some demote this theory (50). Numerous experiments show enhanced appositional bone growth on hydroxyapatite coated implants (29, 115), a greater bone to implant contact (26, 51), implant ingrowth (115) or interface shear strength (37, 56) compared to titanium implants. Fluorapatite has also shown to increase interface shear strength (37, 42) and increase bone contact (15, 26, 31) compared to titanium implants.

Implant surface roughness is another factor associated with micromotion (114). Rough implants are considered to create mechanical interlocking between the bone and the implant (67, 106, 114). An alternative way to improve implant fixation is, as mentioned previously, by systemic treatment (59, 89, 116, 127) of bisphosphonates or local application of bisphosphonates directly on the bone prior to insertion of the prosthesis (58). However, as will be described later, bisphosphonates can not stimulate bone formation directly but inhibit the resorption of already existing bone. Indeed, when Søballe et al. combined the model described above with systemic alendronate treatment, the bisphosphonates were unable to increase the implant fixation when a fibrous tissue layer had already been established in the gap between host bone and implant (108). However, the question whether osseointegration actually has an effect on mechanical implant stability remains to be answered. In 1979, an experiment on dogs showed that fibrous encapsulation was not always associated with non functional implants (22).

Bisphosphonates

What is the bisphosphonate mechanism of action?

Bisphosphonates have affinity to bone mineral. If osteoclasts resorb bone containing bisphosphonate they might become intoxicated and in this way resorption ceases.

Bisphosphonates are used to inhibit resorption of bone in association with various bone diseases such as osteoporosis (46, 76), tumor-associated osteolysis and hyperparathyroidism (28). The chemical structure of the bisphosphonate (*figure 1*) promotes binding to solid-phase calcium phosphates, such as bone mineral. Further, the presence of a hydrogen atom on one side chains (R_1 or R_2 in *fig 1*) is favorable for the affinity (118). As bone resorbing osteoclasts digest the bone, bisphosphonates bound to the bone will become internalized. Depending on the type and dose of bisphosphonate, the bone resorbing capacity will be reduced or the osteoclast might go into apoptosis. The other side group on the bisphosphonate molecule regulates the potency to reduce bone resorption. A nitrogen atom at the end of the other side chain and especially five carbon atoms in a row have shown to be especially efficient. The potency is further increased if other groups are added to the nitrogen atom, as for instance in ibandronate. However, the most potent bisphosphonate known so far is zoledronate.(44)

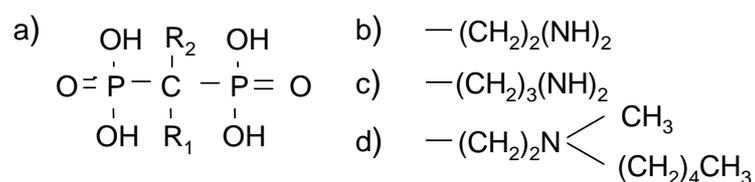


Figure 1. The chemical structure of bisphosphonate (a). R_1 and R_2 signify the bisphosphonate side chains. The chemical structure of the larger side chain for pamidronate (b), alendronate (c) and ibandronate (d).

Nitrogen-containing bisphosphonates are known to inhibit an enzyme, farnesylpyrophosphate synthase, in the mevalonic pathway. As a consequence, less isoprenoid lipids will be formed and the post translational prenylation of certain proteins, such as Ras, Rho, Rac and Rab, will be depressed. Disruption of their function may lead to reduced cell activity or even induce cell death. (45) Inhibition of the mevalonic pathway also leads to accumulation of isopentenyl pyrophosphate and subsequently formation of Appp1 which is able to induce apoptosis by the formation of an ATP- analogue. However, the bisphosphonate itself is not part of the ATP-analogue complex and thus not metabolized. (88)

The mechanism of action for bisphosphonates that do not contain nitrogen is also through the formation of an ATP-analogue but they have no effect on the mevalonic pathway. In contrast to nitrogen-containing bisphosphonates, the non nitrogen-containing bisphosphonate are metabolized. The production of the ATP-analogue causes reduced cell function and may induce cell death. (45, 88)

Do bisphosphonates influence other cells than osteoclasts?

The cell ability to take up bisphosphonates varies. Non resorbing cells might take up bisphosphonates released during osteoclastic bone resorption or due to natural desorption from a bone surface.

Bisphosphonates are naturally released through desorption from bone surfaces or when osteoclasts resorb bisphosphonate containing bone. In the vicinity of a resorption pit the concentration of bisphosphonate may become high since bisphosphonates are transcytosed through the osteoclast which means that the bisphosphonate is released on the opposite side of where it was originally internalized in the osteoclast. There is no doubt that *in vitro* bisphosphonates may be taken up by other cells than osteoclasts and that the protein prenylation is inhibited also in these cells. However, it was shown *in vivo* that the protein prenylation of non resorbing cells was not affected while it was extensively affected in osteoclasts. The suggested explanation was that the cells had a different cell endocytic activity. Consequently, much higher amounts of bisphosphonate might become accumulated in osteoclasts compared to osteoblasts due to the higher endocytic activity. (32)

Besides inhibited bone resorption, other bisphosphonate effects have also been found. An *in vitro* study showed that the bisphosphonate alendronate stimulated activation of cell surviving substances in osteoblasts and thus prevented induction of apoptosis. Binding of the bisphosphonate to the osteoblast membrane resulted in the opening of a hemichannel which ultimately activated the cell surviving substances. (96) However, an *in vivo* study, using a distraction osteogenesis in rabbits, indicated that the systemic treatment with zoledronate did not induce increased osteoblast survival. On the contrary, osteoblast number and surface in the bisphosphonate treated group was lower at 6 weeks after surgery. (107) This might be explained by the coupling between osteoclast and osteoblast during bone remodeling. Thus, a decreased number of osteoclasts could have reduced the osteoblast number as well.

Are bisphosphonates used in orthopaedics to improve implant fixation?

Bisphosphonates have been given orally or applied locally to improve fixation of knee prosthesis and external screw pins.

Radiostereometric analysis showed that the migration of total knee prostheses was reduced when ibandronate was applied directly onto the knee prior to insertion of the prosthesis (58). Oral treatment with Clodronate also showed improved fixation of knee prostheses (59). Proximal bone resorption was reduced around femoral stems after oral treatment with risedronate for 6 months (127). Fixation screws, used to stabilize pertrochanteric fractures in osteoporotic women, showed increased extraction torque in patients that were given alendronate orally (89). Further, one single intravenous infusion of zoledronate increased removal torque of metal pins in patients operated with external fixation pins (116).

What is osteonecrosis of the jaw?

Infections in exposed jaw bones heal poorly in immune compromised patients who have been treated with bisphosphonates for several years. Probably, normal resorption is necessary to eradicate the infection.

A few hundred cases, referred to as osteonecrosis of the jaw, have lately been reported. Nitrogen-containing bisphosphonates are probably incorrectly suspected to be the cause of bone necrosis often appearing at the site of a previous dental trauma (47, 81, 100, 101). In the case of a pathogenic invasion, the antiresorptive effect of the bisphosphonate may result in the establishment of a chronic infection. However, it is still controversial what comes first; the necrotic bone or the infection (10, 55, 81, 101). The anti-angiogenetic characteristics of bisphosphonates was proposed to be the major cause of the observed osteonecrosis and a strong similarity with osteoradionecrosis was suggested (81, 101). However, the fact that living osteocytes are often observed within the lesion supports the hypothesis that infection is a primary and necrosis a secondary effect (10, 55). Another important aspect is that most of the affected patients have been treated with chemotherapeutic agents (81, 101) or radiation therapy (55) of which the latter has shown to reduce the regenerative capacity of the gingival mucosa (30) that otherwise shields the underlying bone and prevents it from being infected. Further, if bisphosphonates have an anti-angiogenetic effect on the bone, this problem should occur in other parts of the body as well. Briefly, the situation is complex and bisphosphonate treatment is one of the contributing factors.

What has been done on bisphosphonate coated implants?

Various kinds of calcium phosphates have been used in many applications to bind bisphosphonates to implants. Bisphosphonates promote integration of implants in bone but a too high dose might perhaps have negative effects.

The information in the following section is shortly presented in *table 1 to 3*.

Bisphosphonates have affinity to calcium which is favorable for binding bisphosphonates to implant surfaces. A number of experiments have been made on implants coated with hydroxyapatite (*table 1*), which is a sort of calcium phosphate

with low solubility (83, 85, 86, 94, 112). A less common method is implanting calcium ions in the implant (64, 128) (*table 3*). Mostly bisphosphonate has been bound to hydroxyapatite or calcium containing implants by incubation in the bisphosphonate solution (35, 36, 64, 83, 85, 86, 94, 95, 112, 128). An alternative method has been to apply droplets of the bisphosphonate solution and then heat evaporate the liquid (48, 112) or filling tube shaped implants with the bisphosphonate solution before insertion (36). In contrast to the hydroxyapatite coatings, entire implants have been made by other types of calcium phosphates (35, 36, 48, 63) (*table 2*). In one study, bisphosphonate was bound to the calcium phosphate and thereby incorporated in the implant (63). Another coating technique was to mix bisphosphonates with a poly(D,L)-lactide matrix that was applied on the implant by dipping it into the matrix solution and subsequently dry it (52). Supersaturation of calcium etidronate was obtained by electrolytic deposition on a titanium implant (*table 3*). When the pH rises near the titanium cathode, etidronate becomes negatively charged, reacts with calcium ions in the solution and is deposited at the cathode.(40)

Table 1. Studies on bisphosphonates bound to implants coated with hydroxyapatite.

<i>Bisphosphonate type & application</i>	<i>Animal or cell</i>	<i>Analysis</i>	<i>Author and year</i>	<i>Bisphosphonate effect</i>
Zoledronate, incubation	Rat (ovex), condyle	Bone density, histomorphometry and pullout	Peter et al 2006	Positive and negative
Zoledronate, incubation	Rat, condyle	Bone mineral density, histomorphometry and pullout	Peter et al 2005	Positive and negative
Zoledronate, incubation or application of droplets	Dog, ulna	Bone ingrowth, bone density, number and size of bone islands	Tanzer et al 2005	Positive
Pamidronate, incubation or mixed with the coating	Osteoclast cell culture	Surface analysis (XPS) and cell staining	McLeod et al 2006	Positive
Alendronate, incubation	Dog, mandible	Bone density	Meraw et al 1999	Positive
Alendronate, incubation	Dog, mandible	Bone implant contact and bone formation rate	Meraw et al 1999	Positive and negative

The response from implants coated with various amounts of zoledronate was examined on rats. However, the following results should be considered with the knowledge that

the analysis was based on mostly three to four replicates or sometimes even 1 or 2. The highest pullout force was obtained for the implant loaded with the third largest amount of bisphosphonate, 2.1 μg , whereas the second largest and largest amount of bisphosphonate, 8.5 and 16.0 μg , showed a decreased pullout force compared to the implant without bisphosphonate (95). When this study was repeated, but on osteoporotic rats, the highest pullout force was obtained for the implant with the second largest amount of bisphosphonate, 8.5 μg (94). Further, the peri-implant bone volume fraction and the bone density were increased within a distance of 200 μm from the implant. (94, 95) However, the study on normal rats showed that the highest amount of bisphosphonate had a negative effect on the bone density within 40 μm from the implant (95).

Table 2. Studies on bisphosphonates bound to implants made of bulk hydroxyapatite.

<i>Bisphosphonate type & application</i>	<i>Animal or cell</i>	<i>Analysis</i>	<i>Author and year</i>	<i>Bisphosphonate effect</i>
Zoledronate, mixed with the coating	Rabbit bone cells on dentin slices	Material analysis (NMR), surface analysis (SEM), resorption activity	Josse et al 2005	Positive
Clodronate, Etidronate & Pamidronate, application of droplets	Rat osteoblasts	Cell viability	Ganguli et al 2002	Positive
Olpadronate, incubation	Rat, tibial metaphysis	Bone mineral density	Denissen et al 2000	No
Olpadronate, incubation & filling the implant with the solution	-	In vitro release	Denissen et al 1994	NA

In another study, porous hydroxyapatite implants were incubated in olpadronate solution with two different pH and inserted in tibial metaphysis in rats. No difference in trabecular mineral bone density and bone mineral density between hydroxyapatite coated implants with and without olpadronate was obtained. (35).

Porous hydroxyapatite coated rod shaped implants, with or without zoledronate, were inserted intramedullary in canine ulna. The relative difference in the fraction of bone that had grown into the porous implant was 134 %, in favor of the zoledronate coated

implant. Further, the size of the bone islands inside the implant was larger for the bisphosphonate coated implants. (112)

Titanium machine-polished or hydroxyapatite coated screws with or without alendronate were inserted in the molars of dogs. The alendronate coated implants showed a 11 % higher bone area density compared to non coated implants (85). This study was repeated but with different measurement techniques. The bone to implant contact obtained for the alendronate coated hydroxyapatite implant was less than the hydroxyapatite implant without bisphosphonate. However, the bone formation rate in the peri-implant area was higher for the alendronate coated hydroxyapatite implant compared to the hydroxyapatite implant without bisphosphonate. (86)

Table 3. Studies on bisphosphonate bound to implants through others methods.

<i>Implant</i>	<i>Bisphosphonate type & application</i>	<i>Animal or cell</i>	<i>Analysis</i>	<i>Author and year</i>	<i>Bisphosphonate effect</i>
Calcium ion implantation	Pamidronate, incubation	Rat, tibia, cortex	Bone formation rate	Kajiwara et al. 2005	Positive
Hydroxyapatite coating & calcium ion implantation	Pamidronate, incubation	Dog, mandible	Bone to implant contact	Yoshinari et al. 2002	Positive
Poly(D,L)-lactide matrix	Zoledronate, mixed with the matrix	Primary human osteoblasts or osteoclast like cells	Cell viability	Greiner et al. 2006 and 2007	Positive
Electrolytic deposition	Etidronate	-	In vitro release, surface analysis	Duan et al. 2004	NA

Cylindrical hydroxyapatite coated implants, with implanted calcium ions, with or without pamidronate were inserted in bone cavities created in the mandibular molar region in dogs. Bisphosphonate coated implants showed to increased the bone to implant contact (128).

Titanium rods with implanted calcium ions with or without pamidronate were inserted in rat tibia. Measurement of the width of newly formed bone, between the implant surface and the medullary cavity, was higher for the pamidronate coated implants after 1, 3 and 4 weeks (64).

A novel coating technique

Adsorbing bisphosphonates to fibrinogen coated implants constituted a novel coating technique. An advantage with this technique is that, in contrast to hydroxyapatite coating techniques, no expensive equipment is needed. The only equipment that was used was an ellipsometer to measure the thickness of the adsorbed molecular layers. Otherwise, the coating technique could be performed in any laboratory. Thus, the biological effects of this coating technique were considered worth further investigating.

MATERIALS AND METHODS

Screws

Stainless steel screws were manufactured of stainless steel (EN:1.4305, ASTM:303, 0.1% C, 1.0% Si, 2.0% Mn, 0.045% P, 0.15-0.345% S, 17-19% Cr, 8-10% Ni). The titanium screws, used in paper II, were fabricated by Nobel Biocare and made of oxidized porous titanium with a similar topography as TiUnite®. The length of the threaded part of the stainless steel and the titanium screws were 2.5 mm and 3 mm and the outer diameter of the threaded part 1.9 mm and 1.7 mm, respectively.

Screw coating

The screw coating technique followed more or less the same procedure in all experiments. However, titanium screws in paper II were cleaned according to a different scheme and the concentration of the fibrinogen solution was 10 times higher in paper I, II and III than in paper IV and in the original article by Tengvall et al (113).

Shortly, silanes were bound to the oxidized metal surface. Glutardialdehyde was bound to the silanes serving as an anchor for fibrinogen attachment. A few hundred Å thick crosslinked fibrinogen matrix was chemically attached to the glutardialdehyde on the metal screw surface. Pamidronate was chemically bound, through the amine group on one side chain, to the carboxylic groups in the fibrinogen matrix, by peptide binding. Subsequently, ibandronate was physically adsorbed to the fibrinogen matrix followed by washing in water, drying and storing the screws in sealed plastic tubes.

Measurements of coating thicknesses

Null ellipsometry, was used to approximate the thickness of the fibrinogen and bisphosphonate layers on the screws. Null ellipsometry only allows measurement on macroscopically and microscopically planar surfaces. Therefore, flat silicon surfaces were used for reference measurements and treated according to the same procedure as the screws, except for the etching during cleaning. Measurements were made at different spots at the surface in order to get a representative estimation of the film thickness.

In situ ellipsometry, allows measurement of very thin molecular layers since the adsorption is measured at exactly the same spot on the surface. It is therefore useful for measurement on less plane surfaces. In paper II bisphosphonates were physically adsorbed to titanium screws. Since thin layers were measured, *in situ* ellipsometry was applied to measure the amount of molecules physically adsorbed to the titanium surfaces.

Mechanical measurements

Pullout measurements

The experimental set up of the pullout experiments is shown in *figure 2*. Sampling started at 0.2 N and was stopped when the curve had dropped to 10 % of the maximal force. Some representative load deformation curves are displayed in *figure 4*. Pullout force signified the maximal force and energy was calculated from the area beneath the load deformation curve. Stiffness was calculated automatically from the slope of the curve but the slope had to be manually specified through the positioning of two marks.



Figure 2. Experimental set up for pullout measurements. The metal hook was connected to a force transducer. The metal plate, through which the screw head points out, was connected to a stepping motor moving down at a speed of 0.2 mm/s.

Removal torque measurements

The experimental set up of the removal torque experiment is shown in *figure 3*. Sampling was started at 0.2 N and was stopped when the screw had turned 90 degrees. Some representative load deformation curves are displayed in *figure 4*. Force was transformed into torque by multiplying with the radius of the cylindrical device. Removal torque at failure signified the maximal torque and energy was calculated from the area beneath the load deformation curve.

Histology

The screw and rat tibia was removed *en bloc*, fixated, dehydrated and embedded in plastic resin. Sections of 15 to 20 μm were prepared by sawing and grinding and were subsequently stained with Toluidine blue. Measurements of the bone to implant contact and bone area density were made with light microscopy. The results consisted of mean values calculated from measurements of four threads on each side of the implant. The percent of bone to implant contact, in each thread, was estimated by the length of contact between bone and implant divided by the perimeter of the thread.

The bone area density was calculated from the ratio between bone area and total area. Measurements were made within the triangle created by a thread and within two rectangles, with the width 250 μm , placed at 0 and 250 μm from the thread edges, respectively.

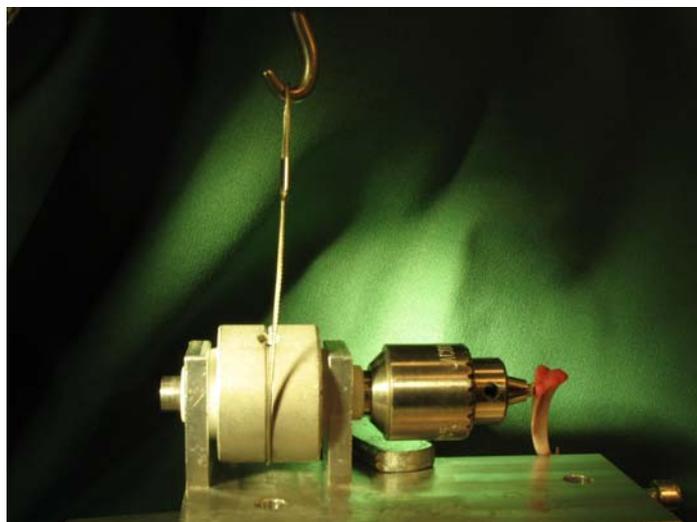


Figure 3. Experimental set up for removal torque measurements. A metal wire was connected to a force transducer and wound around a metal cylinder with a chuck gripping the head of the screw. The cylinder was connected to a stepping motor moving down at a speed of 0.1 mm/s.

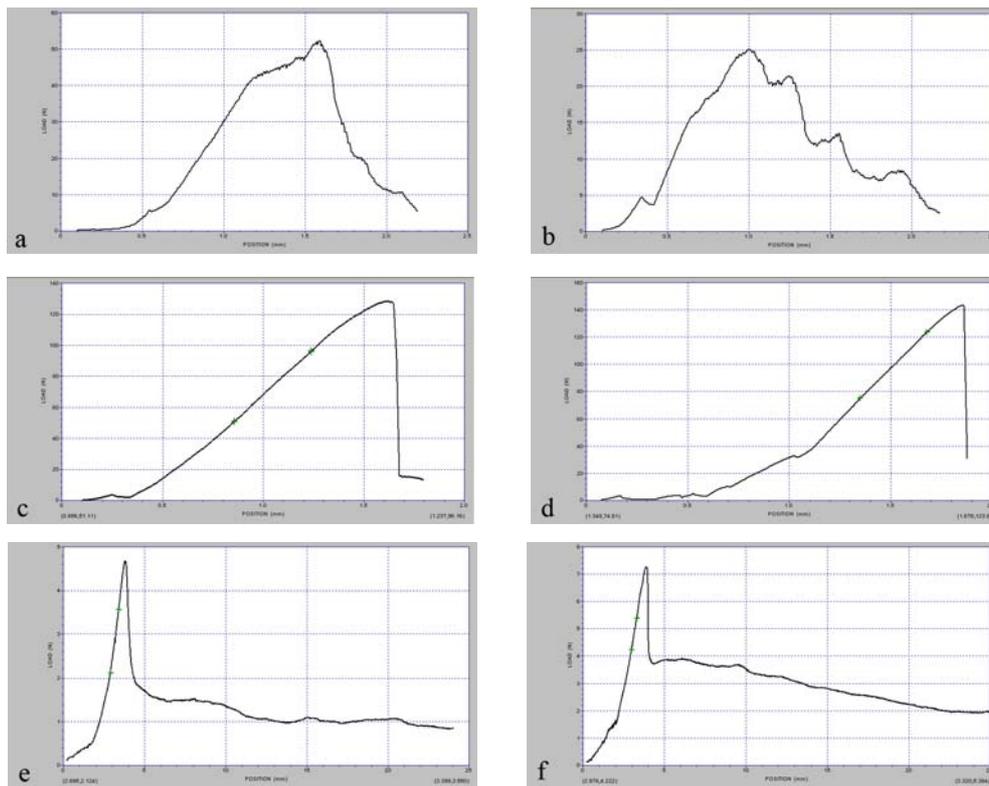


Figure 4. Examples of load deformation curves illustrating pullout (a-d) and removal torque (e-f) measurements. The force (N) is displayed on the y-axis and the displacement (mm) on the x-axis. The irregular curves (a and b) illustrate that the mechanical failure can follow different patterns, which can make up for parts of the biological variation of the results.

RESULTS

Paper I. Surface-bound bisphosphonates enhance screw fixation in rats - increasing effect up to 8 weeks after insertion.

The effect of the treatment was increased with time. The pullout force was higher for bisphosphonate coated screws after 2, 4 and 8 weeks in cortical (*figure 5, left*) and 4 and 8 weeks in cancellous (*figure 5, right*) bone, respectively.

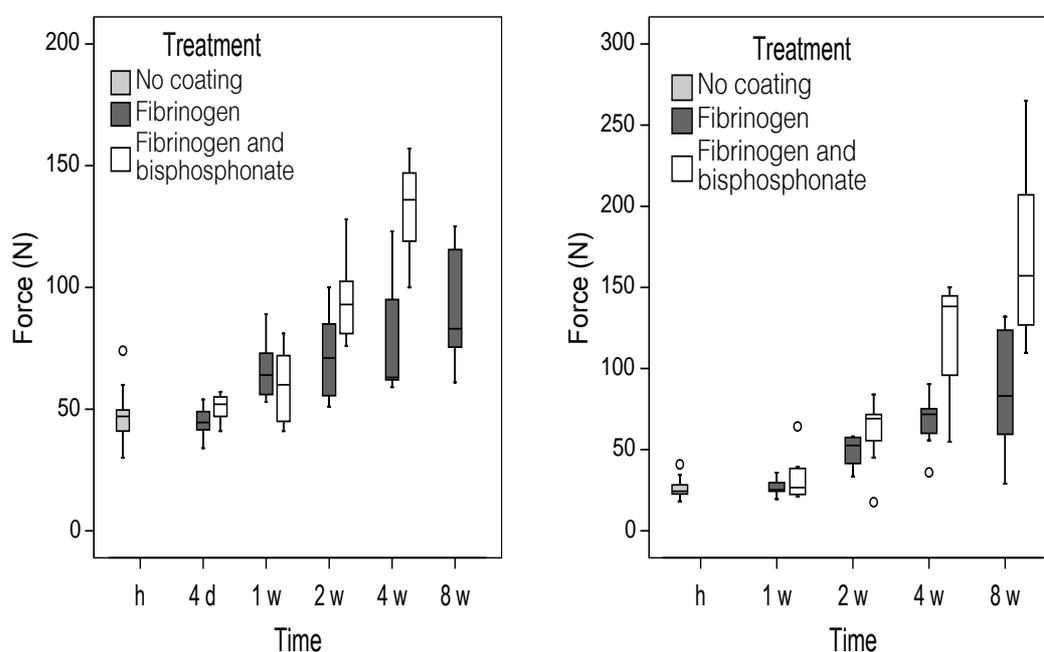


Figure 5. The pullout force for non coated screws directly after insertion, fibrinogen screws and bisphosphonate screws after 4 days (only for cortical bone), 1, 2, 4 and 8 weeks. The screws were inserted in cortical (*left*) and cancellous (*right*) bone.

Paper II. Bisphosphonate coating on titanium screws increases the mechanical fixation in rat tibia after 2 weeks.

The coating technique was applicable, not only on stainless steel but also on commercially pure titanium. The pullout force and energy was higher for bisphosphonate coated screws at 2 weeks, compared to uncoated (*figure 6*) and fibrinogen coated screws. In buffer, approximately 60 % of the bound pamidronate was released within 8 hours (*figure 7*). After 8 days the release leveled off.

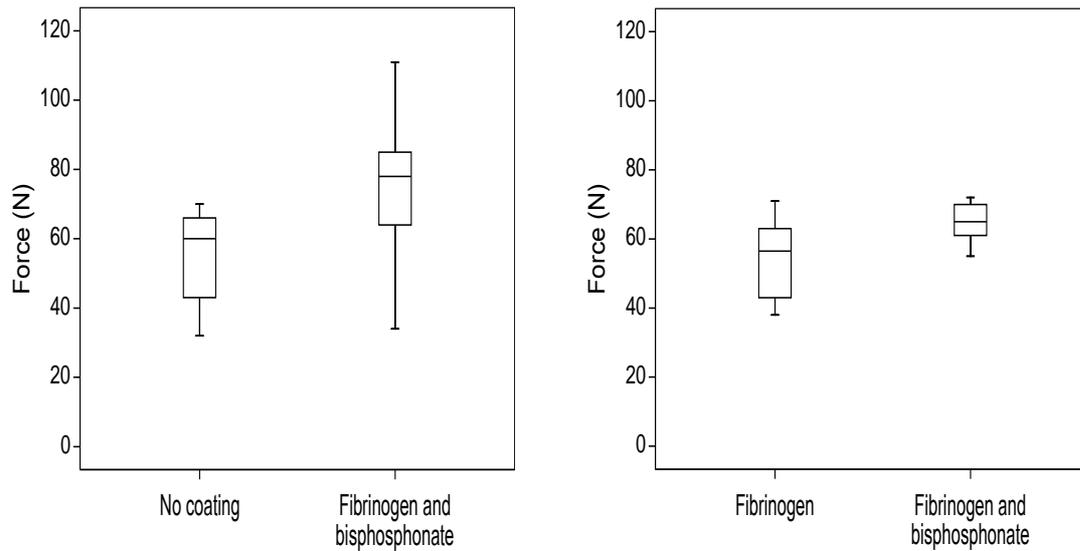


Figure 6. Pullout force for titanium screws with a fibrinogen and bisphosphonate coating. Left; comparison with uncoated control. Right; comparison with fibrinogen coated control.

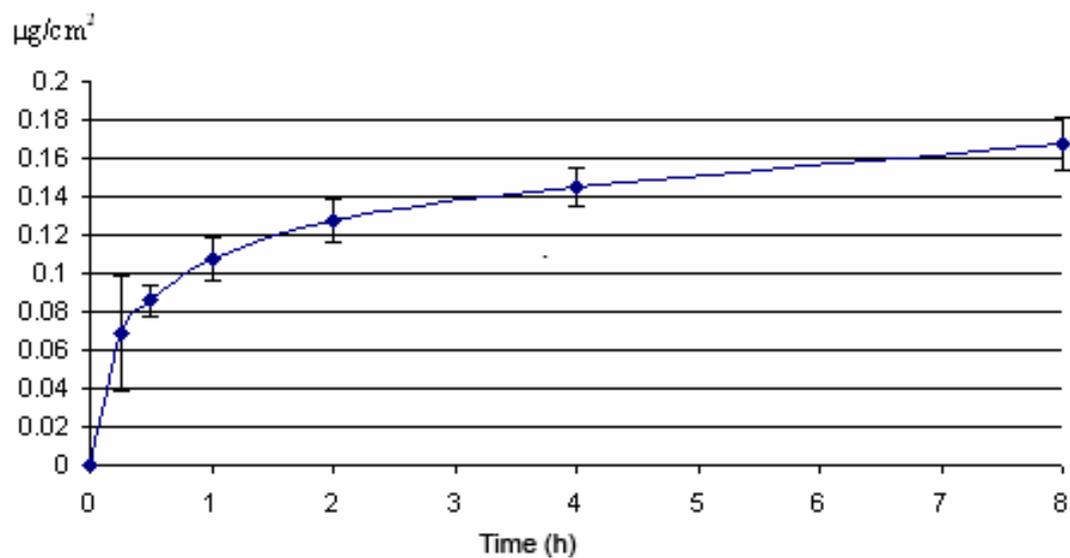


Figure 7. *In vitro*, estimated release of pamidronate covalently linked to fibrinogen coated screws. Numbers are calculated from liquid scintillation measurement of the amount of ^{14}C -alendronate, bound to the screw by the same technique as for pamidronate. The release kinetics was assumed to be equal for alendronate and pamidronate.

Paper III. Stainless steel screws coated with bisphosphonates gave stronger fixation and more surrounding bone. Histomorphometry in rats.

Histomorphometric measurements showed that, at 1 week, the bone to implant contact was higher for the uncoated screws compared to screws with fibrinogen only and screws coated with fibrinogen and bisphosphonates (*figure 8, left*). At 2 weeks the removal torque at failure was higher for bisphosphonate coated screw compared to uncoated and fibrinogen coated controls (*figure 8, right*).

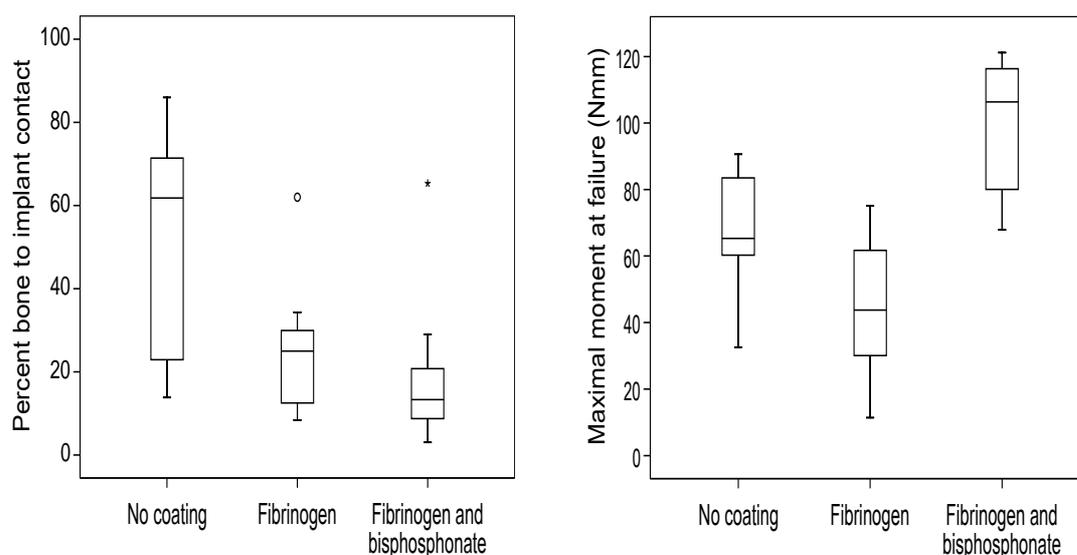


Figure 8. Bone to implant contact in the threads at 1 week (left) and the maximal torque moment at failure at 2 weeks (right).

At 8 weeks there was no difference in bone to implant contact between the uncoated and bisphosphonate coated screws. However, the bone to implant contact was higher for the uncoated and bisphosphonate coated compared to fibrinogen coated screws (*figure 9, left*). The bone area density in the threads after 8 weeks (*figure 9, right*) was higher for bisphosphonate coated screws compared to controls. The results, at 8 weeks, probably reflect the presence of a bone envelope observed around bisphosphonate coated screws (*figure 10, right*) but absent around uncoated screws (*figure 10, left*).

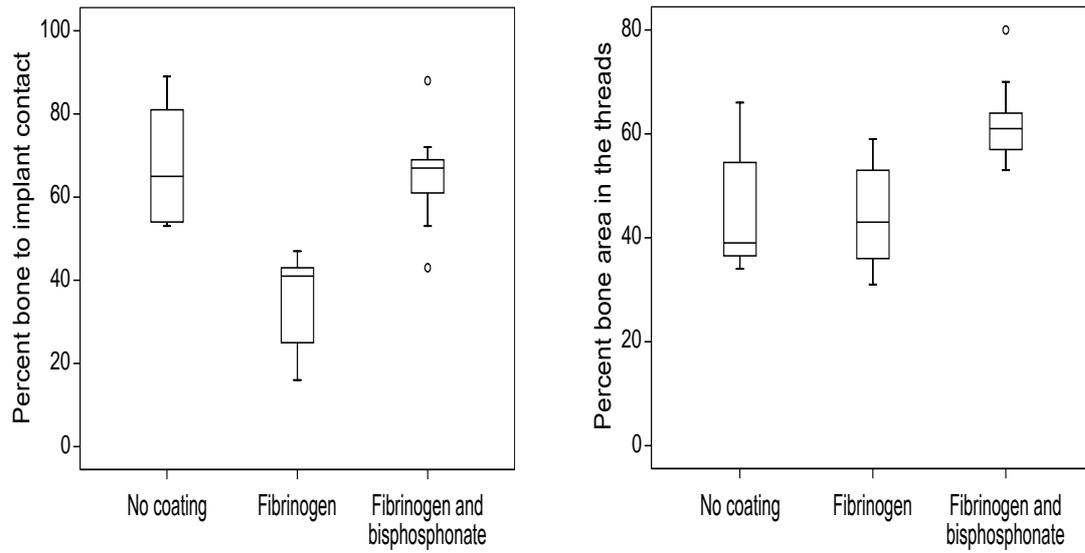


Figure 9. Percent of bone to implant contact (left) and bone area density in the threads at 8 weeks (right).

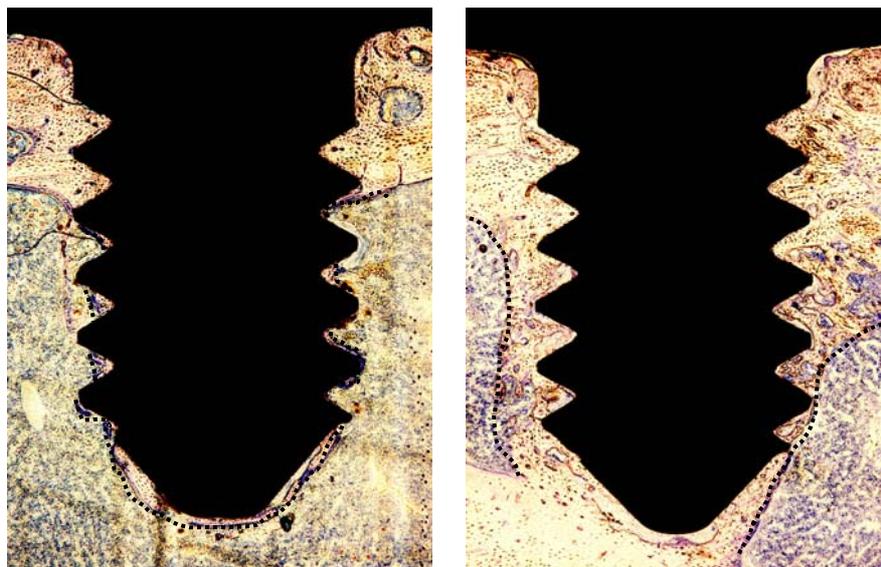


Figure 10. A bone envelope was observed around bisphosphonate coated screws (right) which was absent around the uncoated control screws (left). The dotted lines indicate the border between the bone and the marrow cavity.

Paper IV. A bisphosphonate coating improves the bony fixation of stainless steel screws in ovariectomized rats.

The pullout force and energy was higher for bisphosphonate coated screws (*figure 11*) in ovariectomized rats after 2 weeks, compared to uncoated control screws. Corresponding results in the sham-operated groups were not significant.

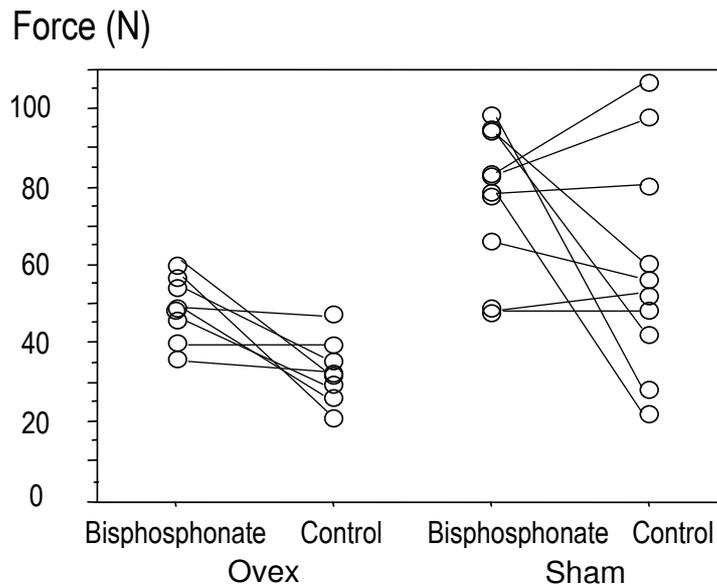


Figure 11. Pullout force for bisphosphonate coated and uncoated screw, inserted bilaterally, pairwise for each rat. Bilateral implants are connected by a line.

DISCUSSION

Methodology

The strength of screw fixation in bone is dependent on the amount and quality of the bone formed around it. Screw shaped implants are designed to transfer axial or transverse loads to the surrounding bone and thus prevent the implant from moving. Pullout measurement is a way of estimating the strength of the bone surrounding the implant (21). In our work, pullout force and energy reflect implant resistance towards axial loading. Pullout stiffness was also reported in paper I and II, but these results were not discussed. The results showed that the stiffness was higher for bisphosphonate compared to fibrinogen control screws and increased with time, independently of the treatment. It is reasonable to suggest that the results were not affected by the elastic properties of the stainless steel screw, since the modulus of elasticity of stainless steel is considerably higher compared to bone. The stiffness probably reflects both the amount and the elastic properties of the bone surrounding the implant. It is unfortunately not possible to distinguish the contribution of each of these factors.

Concerning removal torque, a strong correlation to bone to implant contact has been reported, but a less strong correlation with the bone density 50 μm from the thread edges. (21) Theoretically, removal torque would reflect shearing resistance if there is a chemical bond between bone and implant or mechanical interlocking with the porous implant surface. Otherwise, removal torque could serve as an estimate of the friction between implant and bone surfaces.

Previous studies have shown a reduction in bone to implant contact and removal torque within 4 weeks after insertion in rabbits and rats, respectively (21, 38). In humans, resonance frequency measurements indicated that dental implant stability was reduced between 1 week and 2 to 3 months, depending on the type of implant (49). This phenomenon has been suggested to demonstrate the resorption of bone traumatized during the surgical procedure. In paper I we intended to detect this reduction of bone in order to investigate if the bisphosphonates could inhibit this resorption. However, pullout measurements did not reveal any reduced fixation for the control screws, in cortical or cancellous bone. The pullout measurement might not have been a sufficiently sensitive method or the surgical technique might have been too coarse.

We consider our screw model to be unloaded because the implant has no weight bearing function. However, it is possible that the screw might be exposed to loads sometimes. For example, when the rat is lying down, since the screw head is protruding out of the bone under the skin. An unloaded screw model resembles the circumstances for dental fixtures since these are generally protected from loading the

first 3-6 months (21). Further, the small screw inserted in the rat tibia is mostly in contact with marrow directly after insertion. This might simulate an implant in human cancellous bone, which due to the distance between the trabecula is mostly in contact with marrow.

A great difference between humans and rats is that Haversian systems are rarely seen in rats (60). Anyhow, in both humans and rats a less regular bone structure will prevail until implant stability has been established. Then, the primitive woven bone will be remodeled and reformed into lamellar bone and, depending on the location, into osteonal bone, in humans. Thus this species difference probably has a minor influence on the early events in bone healing around implants.

Another difference between humans and rats is the metabolic activity, which is higher in rats (80). This means that the cellular activity is faster in rats. However, processes such as coagulation at the implant surface or the inflammatory response probably proceed within the same time frame in humans and rats. Concerning bisphosphonates, these will be stored in the bone and exert their effect directly on the osteoclasts after internalization. During resorption of bone containing bisphosphonates, the drug is transported through the osteoclast by transcytosis and released. Thus, it might be bound to bone mineral again or become washed away after the release. (32) Due to the elevated cellular activity, this “recycling” and also the depletion of bisphosphonates is probably faster in rats than in humans.

Preliminary results with radiolabeled bisphosphonate indicate a high amount of bisphosphonate close to the screw surface at 2 weeks. At 8 weeks, the activity was considerably lower, but had spread, so that radioactivity was seen out to about 0.5 mm away from the screw surface. This suggests that the bisphosphonate was initially bound to bone near the implant, but was “recycled” by resorption, so that it could spread to new bone formed further away (*figure 12*).

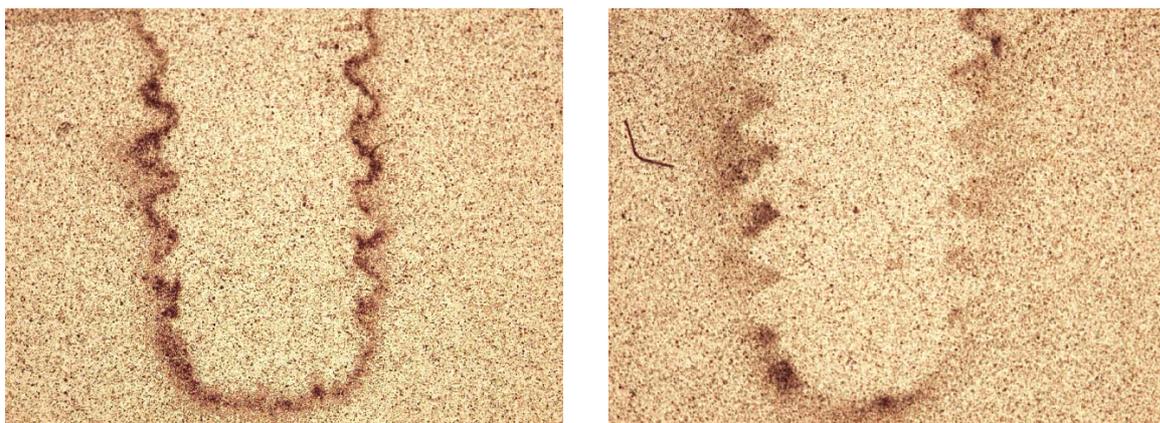


Figure 12. Autoradiography of screws inserted in the tibial metaphysis of rats. The samples were cut trough the screw parallel to the screw axis. Accumulation of ^{14}C -alendronate is represented as darker areas around the screw. In (a), at 2 weeks, bisphosphonate accumulation is seen near the surface. In (b), at 8 weeks, the bisphosphonate is seen at

further distance from the surface, and probably in lower amounts. This might reflect redistribution through “recycling” (se text).

In buffer, more than half of the bisphosphonates bound to the screws were released within 8 hours and almost all bisphosphonates had been released within 8 days (paper II). The bisphosphonate release kinetics is probably similar in rats and humans, but the onset of bone formation or remodeling is probably initiated later in humans than in rats. Thus, the initial distribution of bisphosphonate in the original and newly formed bone might differ between rats and humans.

The bone envelope formed in the rat tibia around the bisphosphonate coated screws seemed to have been formed by apposition of bone growing from the endosteal side of the original cortex and from bony islands created in the medullary cavity adjacent to the implant. The bone extended approximately 300 μm from the thread edges and the depth of the threads is approximately 200 μm . This means that, in rats, a few hundred micrometer of bone was sufficient to encapsulate the implant and improve fixation. However, this might not be mechanically sufficient with larger implants used in humans. It remains to show that the bisphosphonate coating increases the amount of cancellous bone at a larger distance in larger animals.

Pharmacological improvement of implant fixation

Binding bisphosphonates to metal implants through a fibrinogen matrix is a novel coating technique. Other researchers have utilized the bisphosphonate affinity to calcium phosphates or calcium ions. Consequently, there is a lot of literature on bisphosphonates bound to calcium phosphate coated or calcium ion modified implants. A less common technique is to bind bisphosphonates through a poly (D, L)-lactide matrix. This technique has similarities with the fibrinogen coating technique since both shields the underlying metal surface of the implant. Unfortunately, no *in vivo* study on poly (D, L)-lactide matrix technique was found published.

Bisphosphonates exert an anti-catabolic effect on bone, which means that the bone is protected from resorption. When bone formation continues more or less unaffected, the net result is that the bisphosphonates indirectly increase the amount of bone (107). Under these circumstances, the effect of the bisphosphonates is comparable to the effect of bone anabolic drugs. This phenomenon was clearly demonstrated in paper I, where the effect of the bisphosphonate treatment was increased with time and in paper III, where a bone envelope was formed only around bisphosphonate coated screws. At 4 weeks, the biomechanical pullout force of bisphosphonate coated screws (paper I) was more or less in the same range as for rats with uncoated screws treated systemically with a high dose of the anabolic drug parathyroid hormone (62).

Parathyroid hormone is a drug used in patients with severe osteoporosis. It is thought to increase osteoblast number (122) due to enhanced proliferation and differentiation of osteoprogenitor cells (91) or enhanced stimulation of bone lining cells to express the osteoblast phenotype (39, 71). Unfortunately, parathyroid hormone has some

disadvantages. The amount of parathyroid hormone used in the study mentioned above was approximately 40 times larger than the amount that is applicable in humans. Additionally, parathyroid hormone must be given intermittent to yield an anabolic effect and is thus not applicable in association with implant coatings.

There are other potent anabolic drugs, such as the bone morphogenetic proteins, which stimulate differentiation of osteoblasts. They may also produce ectopic bone in muscular tissue through the induction of myogenic cell differentiation. Various studies using bone morphogenetic protein coated implants show enhanced ectopic bone formation when placed in muscle tissue (27, 129) or subcutaneously (53, 123). However, bone morphogenetic proteins also stimulate RANKL-induced osteoclast differentiation *in vivo* (68). *In vitro* studies have shown that some types of bone morphogenetic proteins might stimulate osteoclast formation, recruitment (57) and bone resorption (65). During certain circumstances when formation of osteoclasts is promoted over osteoblast formation, bone morphogenetic proteins can induce bone resorption (77). It has been suggested that a low amount of bone morphogenetic protein should be used to enhance bone to implant contact but a higher amount to enhance porous implant bone ingrowth or bone formation in the gaps around implants (73). Indeed, bone morphogenetic proteins are associated with both negative (3, 78, 110) and positive (72, 73) effects on implant bone integration. Bisphosphonates have been used successfully in combination with bone morphogenetic proteins to inhibit unwanted catabolic responses (61, 77). Unfortunately, bone morphogenetic proteins are very expensive. However, in the future the cost might decrease when certain patents have run out. It would probably be possible to immobilize bone morphogenetic proteins into the fibrinogen matrix. An appropriate amount of bone morphogenetic protein together with the bisphosphonate coating might improve implant fixation further.

Effects of the fibrinogen layer

In our model, at 1 week, the fibrinogen coating appeared to diminish the implant to bone contact (paper III). Further, around screws coated with fibrinogen, trabeculae connecting bone formed at the surface and the surrounding bone seemed to appear more seldom. These observations indicate that the stainless steel surface promoted osseointegration at this early stage, and that this effect was less prominent for screws coated with fibrinogen. One explanation might be that the fibrinogen coating could have shielded the surface and thus diminished these effects. The surface chemistry has been suggested to alter the chemical binding of proteins and cells to the implant surface (19) which is thought affect implant osseointegration (33, 34).

The amount of bone around the implant at 1 week was similar in all groups. However, since the uncoated screws seemed to have a better anchor in the surrounding bone these might have been better fixated at 1 week.

One way to improve implant stability at this early stage would be to retain close contact with cortical bone. As mentioned earlier, the initial implant stability was

suggested to decrease due to resorption of the cortical bone between 1 and 4 weeks in rabbits (38) and rats (21) or after 1 week and 2 to 3 months in humans (49). Necrotic bone containing bisphosphonates is protected from resorption (12, 111). Thus, it is probably possible to retain the original bone with bisphosphonates and improve early implant stability, under the presumption that a delicate surgical technique is used. Histological results in rabbits confirm that resorption of the original cortical bone had begun already at 1 week (38, 104). However, we did not see signs of resorption of the cortical bone around the screws in our model at 1 week. This could mean that bone remodeling had not started. The original cortical bone had been fractured during screw insertion and there were thus only fragments of this bone in close proximity to the screw. Thus, during these circumstances the bisphosphonate would probably only have had minor influences on the implant stability.

The mechanical consequences of our histological findings at 1 week were not measured. However, already at 2 weeks, the removal torque was higher for the bisphosphonate coated screws compared to the uncoated and fibrinogen coated screws. Thus, already at this time point the bisphosphonate coating seems to have improved implant stability in spite of any negative effects of the fibrinogen.

Screws coated with crosslinked ^{125}I -fibrinogen were inserted in tibial metaphysis in rats and twisted out at 2 days and after 2 and 4 weeks. The radioactivity of the screws was subsequently measured. Preliminary results show that approximately 11% of the fibrinogen matrix is released from the screw within the first 2 days and 8-9% within 28 days. This means that *in vivo* approximately 80% of the fibrinogen matrix is still attached to the screw after one month.

Until now, the bone to implant contact has only been discussed from what can be observed with light microscopy. Although we see direct contact in the microscope, this is not the case at the ultrastructural level (8). It remains unclear what effect the fibrinogen coating might have on the bone to implant contact at this level. The consequences of the ultrastructural interface characteristics, for instance on the response towards torque moments, are not known (75). Further, it has been claimed that it is possible that the techniques used during sample preparation for ultrastructural analysis might create artifacts (74).

Stainless steel and titanium

Among orthopaedic surgeons, it is generally agreed that fracture fixation plates made of titanium are more difficult to remove, than plates of stainless steel. The ultrastructural correlate of this observation is that the amorphous interface layer is thicker (6). Probably, the fibrinogen coating on our screws masks or out-weighs this difference. In the long run, however, when the fibrinogen layer has disappeared, it is possible that a titanium surface underneath the coating might be advantageous. Therefore, it was important to confirm that the coating was efficient also on titanium, as was done in paper II.

Estrogen and implant fixation

Not so much is known about the effect of estrogen deficiency on the early tissue response to implants. However, there is some literature on estrogen and fracture healing (18, 25, 70, 84, 90, 117). As is clear from paper I and III, implant fixation involves progressive bone formation around the implant. This formation is a response to the insertion trauma, and can therefore be regarded as fracture healing, or rather as an important part of this complex phenomenon. It is unclear why implant fixation was less in the ovariectomized rat compared to normal controls. There are two possible explanations. Either there was simply less bone at the implantation site, or the fracture healing response was weaker. It is still debated whether the early fracture healing activity is independent of estrogen or not (18, 70, 84, 90). Molecular analysis of tissue adjacent to metal implants showed that the early tissue response to an implant was associated with the upregulation of certain genes. In ovariectomized rats, enhanced expression was only seen for 2 of these 8 genes indicating that ovariectomy-related estrogen deficiency does affect the early tissue response to an implant (92).

We found that the bisphosphonate coating was efficient also in an estrogen deficient situation. This might have some clinical relevance, since the vast majority of fractures occur in osteoporotic bone in elderly people with low sex hormone levels.

Micromotion and hydroxyapatite

Cells adapt to their mechanophysical environment. Thus, if an implant is initially stable, it is more likely that a bone matrix is formed around it. Our experiments were performed on unloaded implants. It remains to see how implant fixation is affected by the bisphosphonate and fibrinogen coating during loading. Concerning micromotion, it has been shown that high amounts of bisphosphonates are needed to prevent bone resorption created by skewing movements between bone and implant (11, 13). Early implant fixation is generally considered to be improved by a hydroxyapatite coating. Although still debated, many researchers now assume that the stimulating effects on bone formation of hydroxyapatite could be explained by the dissolution of the coating (97, 109, 119, 121). Interestingly, during micromotion more hydroxyapatite seems to dissolve. It has been suggested that the increase in dissolution might explain why the hydroxyapatite was particularly efficient for implant fixation when the implant was exposed to micromotion or when mechanical interlocking was absent. (109) If this conclusion is true, it could explain why the hydroxyapatite effect is not always present (17, 29).

An implant with bisphosphonates bound to hydroxyapatite would probably be more tightly fastened in bone. However, this is not always advantageous. External fixation pins need to be removed after some time. This is sometimes difficult with

hydroxyapatite coatings, due to the large surface shear resistance. In this case, bisphosphonates bound to fibrinogen coated screws might be more applicable, because removal torque would only be moderately increased, whereas the surrounding bone would still be stronger.

It is possible that the mechanisms of action of hydroxyapatite and bisphosphonate coatings differ, so that they would be clinically applicable for different indications

Osteonecrosis of the jaw

A few years ago, a “new” disease was described and given the name osteonecrosis of the jaw. The characteristic patient had received nitrogen-containing bisphosphonates, orally or intravenously for several years, had a previous dental trauma and had been treated with chemotherapeutic agents. It has been suggested that the anti-cancer treatment reduced the regenerative capacity of the mucosa which facilitated the invasion of bacteria in the jaw bone (10). With high concentrations of bisphosphonates in the jaw bone, the resorption of the infected bone might become compromised, resulting in a lesion with very poor healing capacity. Treatment with antibiotics and chlorhexidine has been reported sometimes to be successful (103). However, in the case of a bisphosphonate coated implant, in contrast to systemic bisphosphonate treatment, only a defined area surrounding the implant will contain bisphosphonates. Thus, osteonecrosis of the jaw is probably not a problem for bisphosphonate coated implants: in the case of infection, the implant and the bisphosphonate-containing bone around it can be removed.

Future experiments

We intend to investigate the radial distribution of bisphosphonates from the screw surface in cancellous bone of larger species. This is important, since screws that “cut out” of the bone is a problem in e.g. the fixation of pertrochanteric hip fractures. If bisphosphonates were distributed several millimeters out in the bone around the screw, the bone might be prevented from fracturing. A pilot clinical study has recently been conducted on fibrinogen and bisphosphonate coated screws inserted in the maxilla. Preliminary results indicate that these bisphosphonate screws were better fixated compared to the uncoated screws, and a randomized trial has been initiated.

CONCLUSIONS

Adsorbing bisphosphonates to fibrinogen coated implants constitute an alternative binding technique to previous methods. The effects might be different from those using hydroxyapatite for attachment of the bisphosphonate. Hydroxyapatite coating needs expensive equipment and intricate processing (121, 124) in contrast to the fibrinogen method, which is comparatively easy, cheap and quick. Bisphosphonates bound to a fibrinogen coating appear to improve implant fixation.

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I would like to tell my sister (that probably will think this is ridiculous) and her family, that I love you.

And finally

I would like to thank my precious, beloved, Gustaf Ullman 

Poem till Älskling

Som två öar i havet ligger våra hjärtan.

Låt mig komma som en vind över havet
och låta vågorna smeka din strand.

Låt mig komma som en fågel med en olivkvist
i näbben och plantera den på där stranden.

Olivkvisten blir till ett träd; trädet växer,
och ur dess grenar skall du få allt vad du
önskar dig.

Låt trädet växa där i tusen år, men även det
mest livskraftiga träd kommer en dag att vissna.

När så sker, Sörj det ej, utan se trädet med
fågelns ögon, för kärleken är evig och kan
aldrig vissna.

Gustaf Ullman



REFERENCES

1. Abrahamsson, I., Berglundh, T., Linder, E., Lang, N. P., and Lindhe, J. Early bone formation adjacent to rough and turned endosseous implant surfaces. An experimental study in the dog. *Clin Oral Implants Res* 15:381-92; 2004.
2. Adell, R., Eriksson, B., Lekholm, U., Branemark, P. I., and Jemt, T. Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. *Int J Oral Maxillofac Implants* 5:347-59; 1990.
3. Aebli, N., Stich, H., Schawalder, P., Theis, J. C., and Krebs, J. Effects of bone morphogenetic protein-2 and hyaluronic acid on the osseointegration of hydroxyapatite-coated implants: an experimental study in sheep. *J Biomed Mater Res A* 73:295-302; 2005.
4. Albrektsson, T., Branemark, P. I., Hansson, H. A., and Lindstrom, J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. *Acta Orthop Scand* 52:155-70; 1981.
5. Albrektsson, T., Dahl, E., Enbom, L., Engevall, S., Engquist, B., Eriksson, A. R., Feldmann, G., Freiberg, N., Glantz, P. O., Kjellman, O., and et al. Osseointegrated oral implants. A Swedish multicenter study of 8139 consecutively inserted Nobelpharma implants. *J Periodontol* 59:287-96; 1988.
6. Albrektsson, T., and Hansson, H. A. An ultrastructural characterization of the interface between bone and sputtered titanium or stainless steel surfaces. *Biomaterials* 7:201-5; 1986.
7. Albrektsson, T., Hansson, H. A., and Ivarsson, B. Interface analysis of titanium and zirconium bone implants. *Biomaterials* 6:97-101; 1985.
8. Albrektsson, T. O., B, J. C., and L, S. Biological aspects of implant dentistry: osseointegration. *Periodontol* 2000 4:58-73; 1994.
9. Allami, M. K., Fender, D., Khaw, F. M., Sandher, D. R., Esler, C., Harper, W. M., and Gregg, P. J. Outcome of Charnley total hip replacement across a single health region in England. The results at ten years from a regional arthroplasty register. *J Bone Joint Surg Br* 88:1293-8; 2006.
10. Aspenberg, P. Osteonecrosis of the jaw: what do bisphosphonates do? *Expert Opin Drug Saf* 5:743-5; 2006.
11. Aspenberg, P., and Herbertsson, P. Periprosthetic bone resorption. Particles versus movement. *J Bone Joint Surg Br* 78:641-6; 1996.
12. Astrand, J., and Aspenberg, P. Systemic alendronate prevents resorption of necrotic bone during revascularization. A bone chamber study in rats. *BMC Musculoskelet Disord* 3:19; 2002.
13. Astrand, J., and Aspenberg, P. Topical, single dose bisphosphonate treatment reduced bone resorption in a rat model for prosthetic loosening. *J Orthop Res* 22:244-9; 2004.
14. Bellabarba, C., Ricci, W. M., and Bolhofner, B. R. Distraction external fixation in lateral compression pelvic fractures. *J Orthop Trauma* 20:S7-14; 2006.

15. Berglundh, T., Abrahamsson, I., Albouy, J. P., and Lindhe, J. Bone healing at implants with a fluoride-modified surface: an experimental study in dogs. *Clin Oral Implants Res* 18:147-52; 2007.
16. Berglundh, T., Abrahamsson, I., Lang, N. P., and Lindhe, J. De novo alveolar bone formation adjacent to endosseous implants. *Clin Oral Implants Res* 14:251-62; 2003.
17. Berry, J. L., Geiger, J. M., Moran, J. M., Skraba, J. S., and Greenwald, A. S. Use of tricalcium phosphate or electrical stimulation to enhance the bone-porous implant interface. *J Biomed Mater Res* 20:65-77; 1986.
18. Boden, S. D., Joyce, M. E., Oliver, B., Heydemann, A., and Bolander, M. E. Estrogen receptor mRNA expression in callus during fracture healing in the rat. *Calcif Tissue Int* 45:324-5; 1989.
19. Boyan, B. D., Hummert, T. W., Dean, D. D., and Schwartz, Z. Role of material surfaces in regulating bone and cartilage cell response. *Biomaterials* 17:137-46; 1996.
20. Branemark, P. I. Rehabilitation and osseointegration in clinical reality. *Int J Oral Maxillofac Implants* 18:770-1; 2003.
21. Branemark, R., Ohnells, L. O., Nilsson, P., and Thomsen, P. Biomechanical characterization of osseointegration during healing: an experimental in vivo study in the rat. *Biomaterials* 18:969-78; 1997.
22. Brunski, J. B., Moccia, A. F., Jr., Pollack, S. R., Korostoff, E., and Trachtenberg, D. I. The influence of functional use of endosseous dental implants on the tissue-implant interface. I. Histological aspects. *J Dent Res* 58:1953-69; 1979.
23. Burgos, P. M., Rasmusson, L., Meirelles, L., and Sennerby, L. Early Bone Tissue Responses to Turned and Oxidized Implants in the Rabbit Tibia. *Clin Implant Dent Relat Res*; 2008.
24. Burr, D. B., Schaffler, M. B., and Frederickson, R. G. Composition of the cement line and its possible mechanical role as a local interface in human compact bone. *J Biomech* 21:939-45; 1988.
25. Cao, Y., Mori, S., Mashiba, T., Westmore, M. S., Ma, L., Sato, M., Akiyama, T., Shi, L., Komatsubara, S., Miyamoto, K., and Norimatsu, H. Raloxifene, estrogen, and alendronate affect the processes of fracture repair differently in ovariectomized rats. *J Bone Miner Res* 17:2237-46; 2002.
26. Clemens, J. A., Klein, C. P., Sackers, R. J., Dhert, W. J., de Groot, K., and Rozing, P. M. Healing of gaps around calcium phosphate-coated implants in trabecular bone of the goat. *J Biomed Mater Res* 36:55-64; 1997.
27. Cole, B. J., Bostrom, M. P., Pritchard, T. L., Sumner, D. R., Tomin, E., Lane, J. M., and Weiland, A. J. Use of bone morphogenetic protein 2 on ectopic porous coated implants in the rat. *Clin Orthop Relat Res*:219-28; 1997.
28. Coleman, R. E. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. *Cancer Treat Rev* 27:165-76; 2001.
29. Cook, S. D., Thomas, K. A., Kay, J. F., and Jarcho, M. Hydroxyapatite-coated porous titanium for use as an orthopedic biologic attachment system. *Clin Orthop Relat Res*:303-12; 1988.
30. Cooper, J. S., Fu, K., Marks, J., and Silverman, S. Late effects of radiation therapy in the head and neck region. *Int J Radiat Oncol Biol Phys* 31:1141-64; 1995.

31. Cooper, L. F., Zhou, Y., Takebe, J., Guo, J., Abron, A., Holmen, A., and Ellingsen, J. E. Fluoride modification effects on osteoblast behavior and bone formation at TiO₂ grit-blasted c.p. titanium endosseous implants. *Biomaterials* 27:926-36; 2006.
32. Coxon, F. P., Thompson, K., Roelofs, A. J., Ebetino, F. H., and Rogers, M. J. Visualizing mineral binding and uptake of bisphosphonate by osteoclasts and non-resorbing cells. *Bone*; 2008.
33. Davies, J. E. Bone bonding at natural and biomaterial surfaces. *Biomaterials* 28:5058-67; 2007.
34. Davies, J. E. Understanding peri-implant endosseous healing. *J Dent Educ* 67:932-49; 2003.
35. Denissen, H., Martinetti, R., van Lingen, A., and van den Hooff, A. Normal osteoconduction and repair in and around submerged highly bisphosphonate-complexed hydroxyapatite implants in rat tibiae. *J Periodontol* 71:272-8; 2000.
36. Denissen, H., van Beek, E., Lowik, C., Papapoulos, S., and van den Hooff, A. Ceramic hydroxyapatite implants for the release of bisphosphonate. *Bone Miner* 25:123-34; 1994.
37. Dhert, W. J., Klein, C. P., Wolke, J. G., van der Velde, E. A., de Groot, K., and Rozing, P. M. A mechanical investigation of fluorapatite, magnesiumwhitlockite, and hydroxylapatite plasma-sprayed coatings in goats. *J Biomed Mater Res* 25:1183-200; 1991.
38. Dhert, W. J., Thomsen, P., Blomgren, A. K., Esposito, M., Ericson, L. E., and Verbout, A. J. Integration of press-fit implants in cortical bone: a study on interface kinetics. *J Biomed Mater Res* 41:574-83; 1998.
39. Dobnig, H., and Turner, R. T. Evidence that intermittent treatment with parathyroid hormone increases bone formation in adult rats by activation of bone lining cells. *Endocrinology* 136:3632-8; 1995.
40. Duan, K., Fan, Y., and Wang, R. Electrolytic deposition of calcium etidronate drug coating on titanium substrate. *J Biomed Mater Res B Appl Biomater* 72:43-51; 2005.
41. Duncan, R. L., and Turner, C. H. Mechanotransduction and the functional response of bone to mechanical strain. *Calcif Tissue Int* 57:344-58; 1995.
42. Ellingsen, J. E., Johansson, C. B., Wennerberg, A., and Holmen, A. Improved retention and bone-to-implant contact with fluoride-modified titanium implants. *Int J Oral Maxillofac Implants* 19:659-66; 2004.
43. Esposito, M., Hirsch, J. M., Lekholm, U., and Thomsen, P. Biological factors contributing to failures of osseointegrated oral implants. (II). Etiopathogenesis. *Eur J Oral Sci* 106:721-64; 1998.
44. Fleisch, H. Bisphosphonates: mechanisms of action. *Endocr Rev* 19:80-100; 1998.
45. Fleisch, H. Development of bisphosphonates. *Breast Cancer Res* 4:30-4; 2002.
46. Fleisch, H. A. Bisphosphonates: preclinical aspects and use in osteoporosis. *Ann Med* 29:55-62; 1997.
47. Fugazzotto, P. A., Lightfoot, W. S., Jaffin, R., and Kumar, A. Implant placement with or without simultaneous tooth extraction in patients taking oral bisphosphonates: postoperative healing, early follow-up, and the incidence of complications in two private practices. *J Periodontol* 78:1664-9; 2007.

48. Ganguli, A., Henderson, C., Grant, M. H., Meikle, S. T., Lloyd, A. W., and Goldie, I. The interactions of bisphosphonates in solution and as coatings on hydroxyapatite with osteoblasts. *J Mater Sci Mater Med* 13:923-31; 2002.
49. Glauser, R., Lundgren, A. K., Gottlow, J., Sennerby, L., Portmann, M., Ruhstaller, P., and Hammerle, C. H. Immediate occlusal loading of Branemark TiUnite implants placed predominantly in soft bone: 1-year results of a prospective clinical study. *Clin Implant Dent Relat Res* 5 Suppl 1:47-56; 2003.
50. Gottlander, M., and Albrektsson, T. Histomorphometric analyses of hydroxyapatite-coated and uncoated titanium implants. The importance of the implant design. *Clin Oral Implants Res* 3:71-6; 1992.
51. Gottlander, M., Albrektsson, T., and Carlsson, L. V. A histomorphometric study of unthreaded hydroxyapatite-coated and titanium-coated implants in rabbit bone. *Int J Oral Maxillofac Implants* 7:485-90; 1992.
52. Greiner, S., Kadow-Romacker, A., Lubberstedt, M., Schmidmaier, G., and Wildemann, B. The effect of zoledronic acid incorporated in a poly(D,L-lactide) implant coating on osteoblasts in vitro. *J Biomed Mater Res A* 80:769-75; 2007.
53. Hall, J., Sorensen, R. G., Wozney, J. M., and Wikesjo, U. M. Bone formation at rhBMP-2-coated titanium implants in the rat ectopic model. *J Clin Periodontol* 34:444-51; 2007.
54. Handoll, H., Huntley, J., and Madhok, R. Different methods of external fixation for treating distal radial fractures in adults. *Cochrane Database Syst Rev*:CD006522; 2008.
55. Hansen, T., Kunkel, M., Weber, A., and James Kirkpatrick, C. Osteonecrosis of the jaws in patients treated with bisphosphonates - histomorphologic analysis in comparison with infected osteoradionecrosis. *J Oral Pathol Med* 35:155-60; 2006.
56. Hayashi, K., Inadome, T., Mashima, T., and Sugioka, Y. Comparison of bone-implant interface shear strength of solid hydroxyapatite and hydroxyapatite-coated titanium implants. *J Biomed Mater Res* 27:557-63; 1993.
57. Hentunen, T. A., Lakkakorpi, P. T., Tuukkanen, J., Lehenkari, P. P., Sampath, T. K., and Vaananen, H. K. Effects of recombinant human osteogenic protein-1 on the differentiation of osteoclast-like cells and bone resorption. *Biochem Biophys Res Commun* 209:433-43; 1995.
58. Hilding, M., and Aspenberg, P. Local peroperative treatment with a bisphosphonate improves the fixation of total knee prostheses: A randomized, double-blind radiostereometric study of 50 patients. *Acta Orthop* 78:795-9; 2007.
59. Hilding, M., and Aspenberg, P. Postoperative clodronate decreases prosthetic migration: 4-year follow-up of a randomized radiostereometric study of 50 total knee patients. *Acta Orthop* 77:912-6; 2006.
60. Hillier, M. L., and Bell, L. S. Differentiating human bone from animal bone: a review of histological methods. *J Forensic Sci* 52:249-63; 2007.
61. Jeppsson, C., Astrand, J., Tagil, M., and Aspenberg, P. A combination of bisphosphonate and BMP additives in impacted bone allografts. *Acta Orthop Scand* 74:483-9; 2003.

62. Johansson, H. R., Skripitz, R., and Aspenberg, P. Bisphosphonates can block the deterioration in implant fixation after withdrawal of intermittent doses of parathyroid hormone. *J Bone Joint Surg Br* 90:400-4; 2008.
63. Josse, S., Faucheux, C., Soueidan, A., Grimandi, G., Massiot, D., Alonso, B., Janvier, P., Laib, S., Pilet, P., Gauthier, O., Daculsi, G., Guicheux, J. J., Bujoli, B., and Bouler, J. M. Novel biomaterials for bisphosphonate delivery. *Biomaterials* 26:2073-80; 2005.
64. Kajiwara, H., Yamaza, T., Yoshinari, M., Goto, T., Iyama, S., Atsuta, I., Kido, M. A., and Tanaka, T. The bisphosphonate pamidronate on the surface of titanium stimulates bone formation around tibial implants in rats. *Biomaterials* 26:581-7; 2005.
65. Kaneko, H., Arakawa, T., Mano, H., Kaneda, T., Ogasawara, A., Nakagawa, M., Toyama, Y., Yabe, Y., Kumegawa, M., and Hakeda, Y. Direct stimulation of osteoclastic bone resorption by bone morphogenetic protein (BMP)-2 and expression of BMP receptors in mature osteoclasts. *Bone* 27:479-86; 2000.
66. Karrholm, J., Borssen, B., Lowenhielm, G., and Snorrason, F. Does early micromotion of femoral stem prostheses matter? 4-7-year stereoradiographic follow-up of 84 cemented prostheses. *J Bone Joint Surg Br* 76:912-7; 1994.
67. Kasemo, B. Biocompatibility of titanium implants: surface science aspects. *J Prosthet Dent* 49:832-7; 1983.
68. Katagiri, T., and Takahashi, N. Regulatory mechanisms of osteoblast and osteoclast differentiation. *Oral Dis* 8:147-59; 2002.
69. Knutson, K., Lewold, S., Robertsson, O., and Lidgren, L. The Swedish knee arthroplasty register. A nation-wide study of 30,003 knees 1976-1992. *Acta Orthop Scand* 65:375-86; 1994.
70. Kubo, T., Shiga, T., Hashimoto, J., Yoshioka, M., Honjo, H., Urabe, M., Kitajima, I., Semba, I., and Hirasawa, Y. Osteoporosis influences the late period of fracture healing in a rat model prepared by ovariectomy and low calcium diet. *J Steroid Biochem Mol Biol* 68:197-202; 1999.
71. Li, M., Liang, H., Shen, Y., and Wronski, T. J. Parathyroid hormone stimulates cancellous bone formation at skeletal sites regardless of marrow composition in ovariectomized rats. *Bone* 24:95-100; 1999.
72. Lind, M., Overgaard, S., Nguyen, T., Ongpipattanakul, B., Bunger, C., and Soballe, K. Transforming growth factor-beta stimulates bone ongrowth. Hydroxyapatite-coated implants studied in dogs. *Acta Orthop Scand* 67:611-6; 1996.
73. Lind, M., Overgaard, S., Soballe, K., Nguyen, T., Ongpipattanakul, B., and Bunger, C. Transforming growth factor-beta 1 enhances bone healing to unloaded tricalcium phosphate coated implants: an experimental study in dogs. *J Orthop Res* 14:343-50; 1996.
74. Linder, L. Ultrastructure of the bone-cement and the bone-metal interface. *Clin Orthop Relat Res*:147-56; 1992.
75. Linder, L., Albrektsson, T., Branemark, P. I., Hansson, H. A., Ivarsson, B., Jonsson, U., and Lundstrom, I. Electron microscopic analysis of the bone-titanium interface. *Acta Orthop Scand* 54:45-52; 1983.

76. Little, D. G., Cornell, M. S., Briody, J., Cowell, C. T., Arbuckle, S., and Cooke-Yarborough, C. M. Intravenous pamidronate reduces osteoporosis and improves formation of the regenerate during distraction osteogenesis. A study in immature rabbits. *J Bone Joint Surg Br* 83:1069-74; 2001.
77. Little, D. G., McDonald, M., Bransford, R., Godfrey, C. B., and Amanat, N. Manipulation of the anabolic and catabolic responses with OP-1 and zoledronic acid in a rat critical defect model. *J Bone Miner Res* 20:2044-52; 2005.
78. Liu, Y., Huse, R. O., de Groot, K., Buser, D., and Hunziker, E. B. Delivery mode and efficacy of BMP-2 in association with implants. *J Dent Res* 86:84-9; 2007.
79. Malchau, H., Herberts, P., Eisler, T., Garellick, G., and Soderman, P. The Swedish Total Hip Replacement Register. *J Bone Joint Surg Am* 84-A Suppl 2:2-20; 2002.
80. Martignoni, M., Groothuis, G. M., and de Kanter, R. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opin Drug Metab Toxicol* 2:875-94; 2006.
81. Marx, R. E. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. *J Oral Maxillofac Surg* 61:1115-7; 2003.
82. Mason, W. T., Khan, S. N., James, C. L., Chesser, T. J., and Ward, A. J. Complications of temporary and definitive external fixation of pelvic ring injuries. *Injury* 36:599-604; 2005.
83. McLeod, K., Anderson, G. I., Dutta, N. K., Smart, R. S., Voelcker, N. H., Sekel, R., and Kumar, S. Adsorption of bisphosphonate onto hydroxyapatite using a novel co-precipitation technique for bone growth enhancement. *J Biomed Mater Res A* 79:271-81; 2006.
84. Melhus, G., Solberg, L. B., Dimmen, S., Madsen, J. E., Nordsletten, L., and Reinholt, F. P. Experimental osteoporosis induced by ovariectomy and vitamin D deficiency does not markedly affect fracture healing in rats. *Acta Orthop* 78:393-403; 2007.
85. Meraw, S. J., and Reeve, C. M. Qualitative analysis of peripheral peri-implant bone and influence of alendronate sodium on early bone regeneration. *J Periodontol* 70:1228-33; 1999.
86. Meraw, S. J., Reeve, C. M., and Wollan, P. C. Use of alendronate in peri-implant defect regeneration. *J Periodontol* 70:151-8; 1999.
87. Meyer, U., Vollmer, D., Runte, C., Bourauel, C., and Joos, U. Bone loading pattern around implants in average and atrophic edentulous maxillae: a finite-element analysis. *J Craniomaxillofac Surg* 29:100-5; 2001.
88. Monkkonen, H., Ottewill, P. D., Kuokkanen, J., Monkkonen, J., Auriola, S., and Holen, I. Zoledronic acid-induced IPP/ApppI production in vivo. *Life Sci* 81:1066-70; 2007.
89. Moroni, A., Faldini, C., Hoang-Kim, A., Pegreff, F., and Giannini, S. Alendronate improves screw fixation in osteoporotic bone. *J Bone Joint Surg Am* 89:96-101; 2007.
90. Namkung-Matthai, H., Appleyard, R., Jansen, J., Hao Lin, J., Maastricht, S., Swain, M., Mason, R. S., Murrell, G. A., Diwan, A. D., and Diamond, T.

- Osteoporosis influences the early period of fracture healing in a rat osteoporotic model. *Bone* 28:80-6; 2001.
91. Nishida, S., Yamaguchi, A., Tanizawa, T., Endo, N., Mashiba, T., Uchiyama, Y., Suda, T., Yoshiki, S., and Takahashi, H. E. Increased bone formation by intermittent parathyroid hormone administration is due to the stimulation of proliferation and differentiation of osteoprogenitor cells in bone marrow. *Bone* 15:717-23; 1994.
 92. Ozawa, S., Ogawa, T., Iida, K., Sukotjo, C., Hasegawa, H., Nishimura, R. D., and Nishimura, I. Ovariectomy hinders the early stage of bone-implant integration: histomorphometric, biomechanical, and molecular analyses. *Bone* 30:137-43; 2002.
 93. Palmer, S., Fairbank, A. C., and Bircher, M. Surgical complications and implications of external fixation of pelvic fractures. *Injury* 28:649-53; 1997.
 94. Peter, B., Gauthier, O., Laib, S., Bujoli, B., Guicheux, J., Janvier, P., van Lenthe, G. H., Muller, R., Zambelli, P. Y., Bouler, J. M., and Pioletti, D. P. Local delivery of bisphosphonate from coated orthopedic implants increases implants mechanical stability in osteoporotic rats. *J Biomed Mater Res A* 76:133-43; 2006.
 95. Peter, B., Zambelli, P. Y., Guicheux, J., and Pioletti, D. P. The effect of bisphosphonates and titanium particles on osteoblasts: an in vitro study. *J Bone Joint Surg Br* 87:1157-63; 2005.
 96. Plotkin, L. I., Aguirre, J. I., Kousteni, S., Manolagas, S. C., and Bellido, T. Bisphosphonates and estrogens inhibit osteocyte apoptosis via distinct molecular mechanisms downstream of extracellular signal-regulated kinase activation. *J Biol Chem* 280:7317-25; 2005.
 97. Porter, A. E., Hobbs, L. W., Rosen, V. B., and Spector, M. The ultrastructure of the plasma-sprayed hydroxyapatite-bone interface predisposing to bone bonding. *Biomaterials* 23:725-33; 2002.
 98. Prendergast, P. J., Huiskes, R., and Soballe, K. ESB Research Award 1996. Biophysical stimuli on cells during tissue differentiation at implant interfaces. *J Biomech* 30:539-48; 1997.
 99. Robertsson, O., Knutson, K., Lewold, S., and Lidgren, L. The Swedish Knee Arthroplasty Register 1975-1997: an update with special emphasis on 41,223 knees operated on in 1988-1997. *Acta Orthop Scand* 72:503-13; 2001.
 100. Ruggiero, S. L., and Drew, S. J. Osteonecrosis of the jaws and bisphosphonate therapy. *J Dent Res* 86:1013-21; 2007.
 101. Ruggiero, S. L., Mehrotra, B., Rosenberg, T. J., and Engroff, S. L. Osteonecrosis of the jaws associated with the use of bisphosphonates: a review of 63 cases. *J Oral Maxillofac Surg* 62:527-34; 2004.
 102. Ryd, L., Albrektsson, B. E., Carlsson, L., Dansgard, F., Herberts, P., Lindstrand, A., Regner, L., and Toksvig-Larsen, S. Roentgen stereophotogrammetric analysis as a predictor of mechanical loosening of knee prostheses. *J Bone Joint Surg Br* 77:377-83; 1995.
 103. Sawatari, Y., and Marx, R. E. Bisphosphonates and bisphosphonate induced osteonecrosis. *Oral Maxillofac Surg Clin North Am* 19:487-98, v-vi; 2007.

104. Sennerby, L., Thomsen, P., and Ericson, L. E. Early tissue response to titanium implants inserted in rabbit cortical bone. Part II Ultrastructural observation. *J Mater Sci Mater Med*. 4:494-502.; 1993.
105. Sennerby, L., Thomsen, P., and Ericson, L. E. Ultrastructure of the bone-titanium interface in rabbits. *J Mater Sci Mater Med* 3:262-71; 1992.
106. Skripitz, R., and Aspenberg, P. Attachment of PMMA cement to bone: force measurements in rats. *Biomaterials* 20:351-6; 1999.
107. Smith, E. J., McEvoy, A., Little, D. G., Baldock, P. A., Eisman, J. A., and Gardiner, E. M. Transient retention of endochondral cartilaginous matrix with bisphosphonate treatment in a long-term rabbit model of distraction osteogenesis. *J Bone Miner Res* 19:1698-705; 2004.
108. Soballe, K., Chen, X., Jensen, T. B., Kidder, L., and Bechtold, J. E. Alendronate treatment in the revision setting, with and without controlled implant motion: an experimental study in dogs. *Acta Orthop* 78:800-7; 2007.
109. Soballe, K., Hansen, E. S., Brockstedt-Rasmussen, H., and Bunker, C. Hydroxyapatite coating converts fibrous tissue to bone around loaded implants. *J Bone Joint Surg Br* 75:270-8; 1993.
110. Stadlinger, B., Pilling, E., Huhle, M., Mai, R., Bierbaum, S., Scharnweber, D., Kuhlisch, E., Loukota, R., and Eckelt, U. Evaluation of osseointegration of dental implants coated with collagen, chondroitin sulphate and BMP-4: an animal study. *Int J Oral Maxillofac Surg* 37:54-9; 2008.
111. Tagil, M., Aspenberg, P., and Astrand, J. Systemic zoledronate precoating of a bone graft reduces bone resorption during remodeling. *Acta Orthop* 77:23-6; 2006.
112. Tanzer, M., Karabasz, D., Krygier, J. J., Cohen, R., and Bobyn, J. D. The Otto Aufranc Award: bone augmentation around and within porous implants by local bisphosphonate elution. *Clin Orthop Relat Res* 441:30-9; 2005.
113. Tengvall, P., Skoglund, B., Askendal, A., and Aspenberg, P. Surface immobilized bisphosphonate improves stainless-steel screw fixation in rats. *Biomaterials* 25:2133-8; 2004.
114. Thomas, K. A., and Cook, S. D. An evaluation of variables influencing implant fixation by direct bone apposition. *J Biomed Mater Res* 19:875-901; 1985.
115. Thomas, K. A., Cook, S. D., Haddad, R. J., Jr., Kay, J. F., and Jarcho, M. Biologic response to hydroxylapatite-coated titanium hips. A preliminary study in dogs. *J Arthroplasty* 4:43-53; 1989.
116. Tägil, M., W-Dahl, A., Harding, A. K., and Little, D. G. Enhanced pin fixation for un-coated standard pins by a single bisphosphonate infusion in hemicallotaxis osteotomy. A double-blinded placebo controlled randomized study., *IBMS Davos workshop: Bone biology & therapeutics* 42, pp. S1-116. Davos, Switzerland: Bone; 2008.
117. Walsh, W. R., Sherman, P., Howlett, C. R., Sonnabend, D. H., and Ehrlich, M. G. Fracture healing in a rat osteopenia model. *Clin Orthop Relat Res*:218-27; 1997.
118. van Beek, E. R., Lowik, C. W., Ebetino, F. H., and Papapoulos, S. E. Binding and antiresorptive properties of heterocycle-containing bisphosphonate analogs: structure-activity relationships. *Bone* 23:437-42; 1998.

119. van Blitterswijk, C. A., Grote, J. J., Kuypers, W., Blok-van Hoek, C. J., and Daems, W. T. Bioreactions at the tissue/hydroxyapatite interface. *Biomaterials* 6:243-51; 1985.
120. Van der Vis, H. M., Aspenberg, P., Marti, R. K., Tigchelaar, W., and Van Noorden, C. J. Fluid pressure causes bone resorption in a rabbit model of prosthetic loosening. *Clin Orthop Relat Res*:201-8; 1998.
121. Wang, H., Eliaz, N., Xiang, Z., Hsu, H. P., Spector, M., and Hobbs, L. W. Early bone apposition in vivo on plasma-sprayed and electrochemically deposited hydroxyapatite coatings on titanium alloy. *Biomaterials* 27:4192-203; 2006.
122. Watson, P. H., Fraher, L. J., Kisiel, M., DeSousa, D., Hendy, G., and Hodsman, A. B. Enhanced osteoblast development after continuous infusion of hPTH(1-84) in the rat. *Bone* 24:89-94; 1999.
123. Vehof, J. W., Mahmood, J., Takita, H., van't Hof, M. A., Kuboki, Y., Spauwen, P. H., and Jansen, J. A. Ectopic bone formation in titanium mesh loaded with bone morphogenetic protein and coated with calcium phosphate. *Plast Reconstr Surg* 108:434-43; 2001.
124. Wen, J., Leng, Y., Chen, J., and Zhang, C. Chemical gradient in plasma-sprayed HA coatings. *Biomaterials* 21:1339-43; 2000.
125. Wennerberg, A., Albrektsson, T., Andersson, B., and Krol, J. J. A histomorphometric and removal torque study of screw-shaped titanium implants with three different surface topographies. *Clin Oral Implants Res* 6:24-30; 1995.
126. Virdee, P., and Bishop, K. A review of the aetiology and management of fractured dental implants and a case report. *Br Dent J* 203:461-6; 2007.
127. Yamasaki, S., Masuhara, K., Yamaguchi, K., Nakai, T., Fuji, T., and Seino, Y. Risedronate reduces postoperative bone resorption after cementless total hip arthroplasty. *Osteoporos Int* 18:1009-15; 2007.
128. Yoshinari, M., Oda, Y., Inoue, T., Matsuzaka, K., and Shimono, M. Bone response to calcium phosphate-coated and bisphosphonate-immobilized titanium implants. *Biomaterials* 23:2879-85; 2002.
129. Zhang, R., Xu, D., Landeryou, T., Toth, C., Dimaano, N., Berry, J., Evans, J., and Hawkins, M. Ectopic bone formation using osteogenic protein-1 carried by a solution precipitated hydroxyapatite. *J Biomed Mater Res A* 71:412-8; 2004.
130. Zioupos, P., and Currey, J. D. The extent of microcracking and the morphology of microcracks in damaged bone. *J Mater Sci* 29:978-86; 1994.