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# IMPLANTS DELIVERING BISPHOSPHONATE LOCALLY INCREASE PERIPROSTHETIC BONE DENSITY IN AN OSTEOPOROTIC SHEEP MODEL. A PILOT STUDY

V. A. Stadelmann[1], O. Gauthier[2], A. Terrier[1], J.-M. Bouler[2], D. P. Pioletti[1*]

[1]Laboratory of Biomechanical Orthopedics EPFL-DAL, IBI, Ecole Polytechnique Fédérale de Lausanne, Switzerland
[2]Laboratoire de Recherche sur les Matériaux d'Intérêt Biologique INSERM 99-03, Faculté de Chirurgie Dentaire, Nantes, France

## Abstract

It is a clinical challenge to obtain a sufficient orthopaedic implant fixation in weak osteoporotic bone. When the primary implant fixation is poor, micromotions occur at the bone-implant interface, activating osteoclasts, which leads to implant loosening. Bisphosphonate can be used to prevent the osteoclastic response, but when administered systemically its bioavailability is low and the time it takes for the drug to reach the periprosthetic bone may be a limiting factor. Recent data has shown that delivering bisphosphonate locally from the implant surface could be an interesting solution. Local bisphosphonate delivery increased periprosthetic bone density, which leads to a stronger implant fixation, as demonstrated in rats by the increased implant pullout force. The aim of the present study was to verify the positive effect on periprosthetic bone remodelling of local bisphosphonate delivery in an osteoporotic sheep model. Four implants coated with zoledronate and two control implants were inserted in the femoral condyle of ovariectomized sheep for 4 weeks. The bone at the implant surface was 50% higher in the zoledronate-group compared to control group. This effect was significant up to a distance of 400μm from the implant surface. The presented results are similar to what was observed in the osteoporotic rat model, which suggest that the concept of releasing zoledronate locally from the implant to increase the implant fixation is not species specific. The results of this trial study support the claim that local zoledronate could increase the fixation of an implant in weak bone.

**Keywords**: orthopaedic implant, drug delivery *in vivo*, sheep, bisphosphonate coating

*Address for correspondence:
Dominique P. Pioletti
Laboratory of Biomechanical Orthopedics EPFL-DAL
Bat AI – Station 15 - EPFL
CH 1015 Lausanne
Switzerland

Telephone Number: +41 21 693 83 41
FAX Number: +41 21 693 86 60
E-mail: dominique.pioletti@epfl.ch

## Introduction

A current clinical challenge in the field of orthopaedics is to obtain a stable implant fixation in weak osteoporotic bone. The fixation of orthopaedic implants in bone relies strongly upon the initial stability of the implant. When the initial stability is not achieved, micromotions occur at the bone implant interface (Mandell *et al.*, 2004; Ramaniraka *et al.*, 2000). The micromotions then activate an osteoclastic response (Stadelmann *et al.*, 2008), which results in periprosthetic osteolysis and later implant migration and wear (Karrholm *et al.*, 1994). Both particulate formation from implant wear and implant migration have been shown to be associated with increased implant failure rate (Clarke *et al.*, 1992; Horikoshi *et al.*, 1994). In the case of osteoporotic patients, this early phase is particularly delicate as the bone is already weak at the time of surgery. In this case, the resorption of a small amount of bone near the implant may induce a dramatic decrease in early fixation, accelerating the failure process.

Bisphosphonate can be used to reduce periprosthetic osteolysis allowing orthopaedic implants to achieve a stronger primary fixation (Hilding *et al.*, 2000). Bisphosphonate molecules inhibit osteoclastic activity, and therefore are widely used to treat patients with osteoporosis (Bone *et al.*, 2004; Fleisch, 2002). However, when administered orally, the bioavailability of bisphosphonate is generally very low, and its local delivery can be further delayed in regions of the skeleton with low blood perfusion, for example the femoral neck. Recent clinical studies have shown that systemic bisphosphonate treatment following prosthesis implantation reduced periprosthetic bone loss only after 3 months (Nehme *et al.*, 2003; Venesmaa *et al.*, 2001b), while significant bone loss arises during this initial period of 3 months (Venesmaa *et al.*, 2001a). A solution to accelerate the local availability of bisphosphonate at the implant location is to deliver the drug locally. This insures the immediate presence of drug molecules at the implant location regardless of the local blood perfusion.

Implants with local delivery of bisphosphonate have been studied previously in small animal models and the results are generally encouraging: In rats, hydroxyapatite coated implants releasing zoledronate increased periprosthetic bone density and the pullout force (Peter *et al.*, 2005); fibrin coated cortical screws releasing ibandronate and pamidronate increased the pullout force (Wermelin *et al.*, 2007). Moreover, in dog ulna, Tanzer *et al*. showed that local elution of zoledronate can cause

substantial bone augmentation around and within porous tantalum implants (Tanzer *et al.*, 2005). Concerning the osteoporotic bone case, hydroxapatite coated implants releasing zoledronate increased pullout force and periprosthetic bone density, compared to control implants in osteoporotic rats (Peter *et al.*, 2006).

Many differences, such as mineral density, healing capacity or the response to mechanical stimuli, exist in the bone metabolism of small animals when compared to that of humans (Egermann *et al.*, 2005; Holy *et al.*, 2000). Therefore it is questionable to extrapolate the *in vivo* results of small animals into the specific clinical situation of osteoporotic patients without pre-clinical tests in a large animal (Buma *et al.*, 2004). To our knowledge, no data exists concerning local delivery of bisphophonate to increase fixation strength of an implant in a large osteoporotic animal model.

Therefore, the aim of the present study was to verify the efficacy of local bisphosphonate delivery to increase the periprosthetic bone density in an osteoporotic sheep model (Turner, 2002).

## Materials and Methods

### Implants

Six Titanium alloy (TA6V) cylinders (diameter 3 mm; length 5 mm) were plasma-coated with hydroxyapatite (thickness: 20 µm; crystallinity index 62%). Two samples were used as controls, while the remaining 4 samples were soaked for 48h in 5ml ultrapure water solutions of 2.25 $10^{-5}$ mol $L^{-1}$ of Zoledronate (1-hydroxy-2-[(1H-imidazole-1-yl)ethylidene] 1-bisphosphonic acid disodium salt) supplied by Novartis Pharmaceuticals AG, Basel, Switzerland. The amount of zoledronate loaded onto the implants was calculated to be 2.1 µg for each implant (Josse *et al.*, 2004).

### Animals and surgical procedures

Animal handling and surgical procedures were conducted according to the European Community Guidelines for the care and use of laboratory animals (DE 86/609/CEE) and approved by the local ethical committee at the Nantes Veterinary School. Three adult female vendeen sheep with an average body weight of 55 kg were used in this study. Six months prior to the study, animals had been neutered by ovariectomy to induce osteoporosis. Subsequent bone changes were investigated on iliac crest bone biopsies. A control biopsy was harvested on the day of ovariectomy and was compared with a bone sample from the contralateral iliac crest harvested 6 months later on the day of implantation. Changes in the microarchitecture of iliac crest biopsies were investigated through 3D microtomography analysis.

The tested cylinders were implanted bilaterally for 4 weeks at the distal femoral end of the 3 mature female sheep. The first animal had two control implants. The second and third animals had two zoledronate coated implants. After 2 weeks of acclimatization, general anaesthesia was induced using an intravenous injection of 4 mg/kg of propofol (Rapinovet®, Schering-Plough, Levallois-Perret, France) and 0.1 mg/kg of diazepam (Valium®, Roche, Neuilly-sur-Seine, France). Anaesthesia was maintained for surgery with a gas mixture of isoflurane (1.5 %), and oxygen (98.5 %). A single dose of morphine (0.5 mg/kg) was injected subcutaneously at the beginning of the surgery as an analgesic. After shaving and disinfection (Vetedine®, Vetoquinol, Lure, France) of the knee, a stifle arthrotomy was performed to expose the distal lateral condyle of the femur.

A cylindrical osseous defect (3 mm in diameter and 6 mm length) was created on the distal femoral epiphysis using a motor-driven drill (Aesculap, Tuttlingen, Germany). After saline irrigation, the osseous cavity was carefully dried and filled with the coated cylinder. The joint capsule was closed with non-absorbable sutures (Prolene® 2-0, Ethicon, Issy Les Moulineux, France). The subcutaneous tissues and skin were closed in different layers using absorbable sutures (Polysorb® 2-0, TycoHealthcare, Elancourt, France). Finally, the surgical wound was covered with an adhesive bandage. Both hind limbs were operated, giving 2 tested implants per animal. At the end of surgery, animals received an injection of meloxicam to complete analgesia (Métacam Bovin®, Boehringer Ingelheim, Germany) but did not receive any postoperative antibiotics. The procedure was repeated on the contra lateral side.

After 4 weeks, general anaesthesia was induced by a mixed injection of ketamine (Imalgène®1000, Merial, Lyon, France) and xylazine (Rompun®, Bayer, Puteaux, France) and the animals were euthanatized by intravenous injection of 20 ml of pentobarbital (Doléthal®, Vétoquinol) through a catheter placed into the jugular vein. The femoral extremities were then dissected from the surrounding soft tissues and immediately placed in a 10 % neutral formol solution.

### Preparation for imaging

The femoral distal ends were then immediately dissected, fixed in glutaraldehyde solution, and stored in a 4% paraformaldehyde, 0.1% glutaraldehyde in 0.08 M cacodylate buffer. Using a handsaw, the condyle was sawed off 1 cm above the implant. The sample was dehydrated in a series of alcohol solutions. The first impregnation step was to soak the sample in a mixture of 50% alcohol 1008 and 50% methyl methacrylate MMA (Fluka Chemika, Sigma Aldrich Chemie Gmbh, Steinheim, Germany) during 24 hours. The second impregnation step was to soak the sample in pure MMA during 24 hours. The first inclusion step was to soak the dehydrated sample during 2 hours under vacuum in a solution containing 90% MMA, 10% dibutylphtalate (Fluka Chemika) and 1% benzoyl peroxide (Fluka Chemika). The sample was then removed from the solution and soaked in the same solution but enhanced by a polymerization activator (N,N-dimethylp-toluidine) (Fluka Chemika). The polymerization took place at -20°C and was complete after 48 hours. Two to four slices of 300µm thick were cut from each sample, using a Microtome 1600 (Leica, Nussloch, Germany) diamond saw. The cutting plane was perpendicular to the implant.

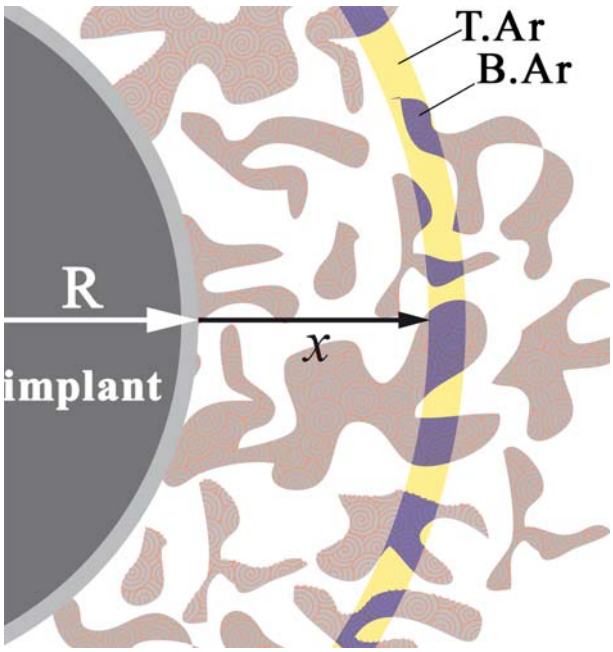


**Figure 1**: Schematic of the method used to determine the bone surface fraction profile. At a distance *x* from the coating surface, a region of interest (ROI) was defined as an annulus of 20µm thickness co-centred with the implant of radius *R*+*x*, where *R* is the implant radius. Then the number of bone pixels inside the ROI (blue pixels) was divided by the total number of pixels in the ROI (blue+yellow pixels). The distance *x* was incremented from 0 to 1mm in steps of 20µm.

### Scanning Electron Microscopy

Slices were carbon-coated and observed using a JEOL JSM 6300 scanning electron microscope (SEM) (JEOL, Tokyo, Japan) using the backscattered electron detector, allowing mineralized bone to be distinguished from soft tissue.

Then, SEM images were used to measure the bone density as a function of the distance from the coating. The implant surface and trabecular bone regions were defined manually on each image. The implant centre and radius were then calculated by least square fitting of a circle onto the implant surface. A threshold was applied to the image in order to distinguish bone from other tissues: pixels with a gray level between 0 and 62 were considered as calcified bone, while those with a gray level from 63 to 255 were considered as other tissues. We defined successive regions of interests inside the trabecular bone in the form of series of ten 20µm thick arcs co-centred with the implant. In each arc, the number of bone pixels was counted and the bone surface fraction (B.Ar/T.Ar) was defined as bone pixels divided by total pixels in the arc, using custom algorithms developed with *Image Processing* for *Mathematica* (Fig. 1).

**Table 1**: Number of animals, implants and slices per group

| | Control | Zoledronate |
|---|---|---|
| **Sheep** | 1 | 2 |
| **Implants** | 2 | 4 |
| **Slices** | 6 | 13 |

### Statistics

The number of slices per group was accounted for as repetition of the density measurement of the same group. Student *t*-test was used to determine the statistical significance of the results. Wilcoxon test was used to identify the significant changes of microstructural properties in the iliac crest biopsies.

## Results

A total of 19 slices (6 control, 13 zoledronate-loaded) were processed (Table 1).

### Osteoporosis induction

Significant microstructural evolutions were measured on the iliac crest biopsies (Fig. 2). The bone volume fraction (BV/TV) decreased by 30% at six months post-ovariectomy. While trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp) decreased by 11%, 19%, and increased by 14% respectively; the structural model index (SMI) was not significantly affected (Table 2).

### Implant integration

The implant integration was verified qualitatively during backscattered electron microscopy imaging. We observed homogenous bone-implant contact as well as new bone formation along the coating surface. Lacunae were observed in the bone speckles growing from the coating. Different levels of mineralization were observed in the newly formed bone. The coating was partially resorbed, and new bone was observed in the resorption zones (fig. 3).

**Table 2**: Microstructure data of iliac crest biopsies obtained before ovariectomy (Control) and the day of implantation (OVX), data as Mean±SD. The evolution column shows significant changes ($p<0.05$). The relative evolution of bone architecture parameters emphasises osteoporosis induction.

| | Control | OVX | Evolution (%) |
|---|---|---|---|
| **BV/TV (%)** | 19.0 ± 3.3 | 13.3 ± 1.3 | -30 |
| **Tb.Th (µm)** | 144.1 ± 15.8 | 128.0 ± 11.1 | -11 |
| **Tb.N ($10^{-3}$/µm)** | 1.32 ± 0.14 | 1.07 ± 0.15 | -19 |
| **Tb.Sp (µm)** | 683 ± 56 | 783 ± 66 | +14 |
| **SMI** | 0.86 ± 0.23 | 0.90 ± 0.18 | |

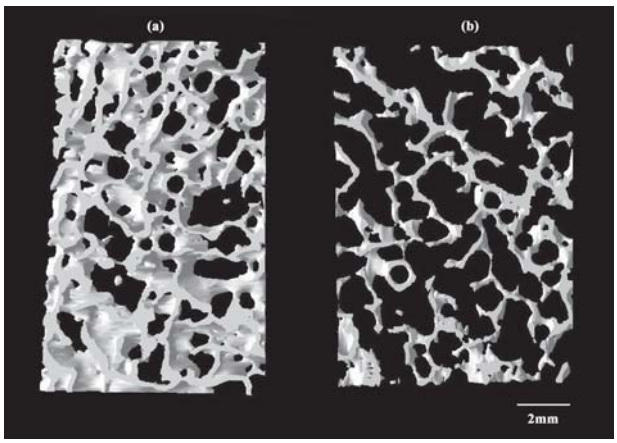


**Figure 2**: Three-dimensional microtomographic description of iliac crest biopsies microarchitecture (a) before ovariectomy and (b) 6 months after ovariectomy. The difference illustrates the induction of osteoporosis.

**Bone surface fraction**

The bone surface fraction (B.Ar/T.Ar) in a 20µm-thick layer around the implant was 50% higher in the zoledronate group compared to the control group (B.Ar/T.Ar=0.45±0.08 for the zoledronate group, 0.30±0.07 for the control group, $p<0.05$). B.Ar/T.Ar of the zoledronate group decreased from 0.45±0.08 at the coating surface to 0.30±0.05 at 800µm from the coating, while the profile of the control group was almost constant (figure 4a). The difference between B.Ar/T.Ar of the zoledronate group and the control group was significant up to 400µm from the coating (figure 4b). The total bone area (B.Ar) in 400µm-thick layer around the implant was 10% greater in zoledronate group compared to control group ($p<0.05$) (fig.4c).

## Discussion

Implants locally delivering bisphosphonate have been shown to increase periprosthetic bone density and pullout forces in previous studies using rat models (Kajiwara *et al.*, 2005; Wermelin *et al.*, 2007). However, the bone metabolism of healing and remodelling in rats is different in humans (Buma *et al.*, 2004; Egermann *et al.*, 2005). Therefore, a large animal study was necessary to validate these results for later applications to human patients with osteoporotic bone. In this study, our aim was to use the osteoporotic sheep model to verify the efficacy of locally delivering zoledronate from an orthopaedic implant to increase the periprosthetic bone density.

Our results showed that B.Ar/T.Ar in a 20µm layer was increased by 50% in the locally delivered-zoledronate group compared to the control group in osteoporotic sheep. The effect of local delivery of zoledronate on B.Ar/T.Ar was significant up to 400µm distance from the implant. The mean B.Ar/T.Ar in the 400µm region around the implant was increased by 10% in the zoledronate-group compared to the control group.

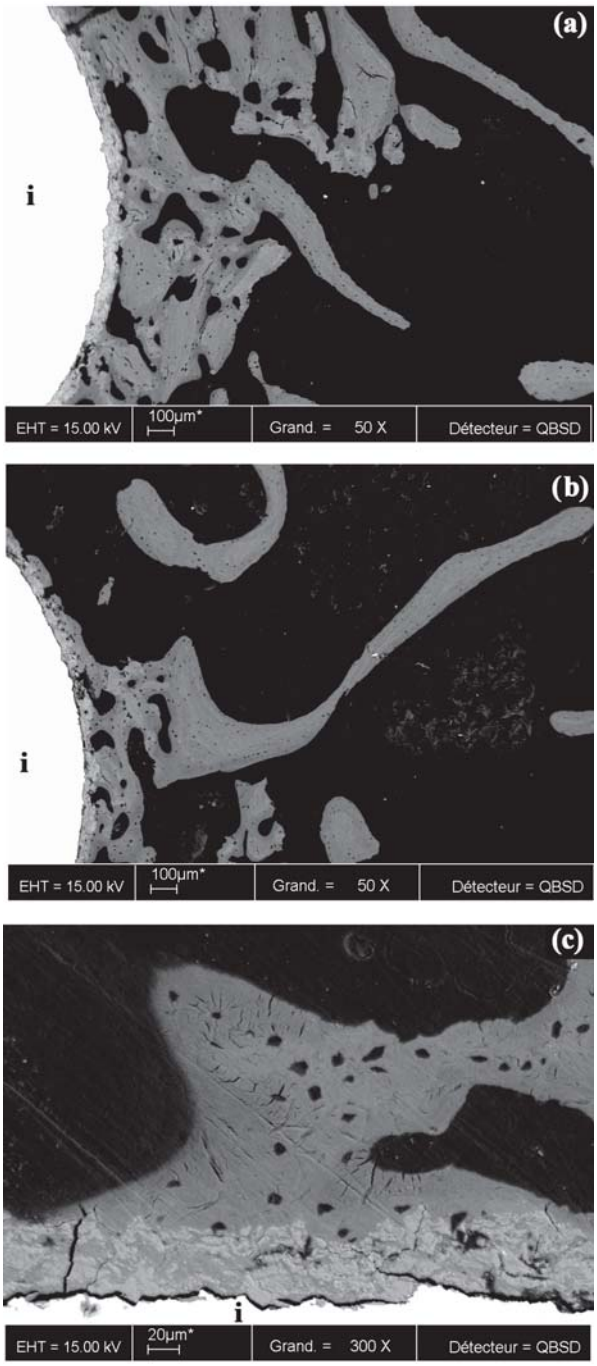


**Figure 3**: SEM pictures of three slices of implants; **(a)** Local bone structure of a condyle implanted with an HA-coated implant (i) containing 2.1µg zoledronate; **(b)** Local bone structure of a condyle implanted with a control implant (i); **(c)** Details of the osseointegration of an zoledronate-implant (i) shows bone growth into resorbed spaces of the coating. Osseointergation in control implants was qualitatively similar.

The implant and dose of zoledronate used in the sheep condyles are identical to those implanted for three weeks in previous rat and OVX rat models (Peter *et al.*, 2005, 2006). The experimental timeline for the sheep was chosen to be four weeks as to compensate for the possibility of a slower remodelling rate in larger animals. When the results of these different models were compared (Table 2), we

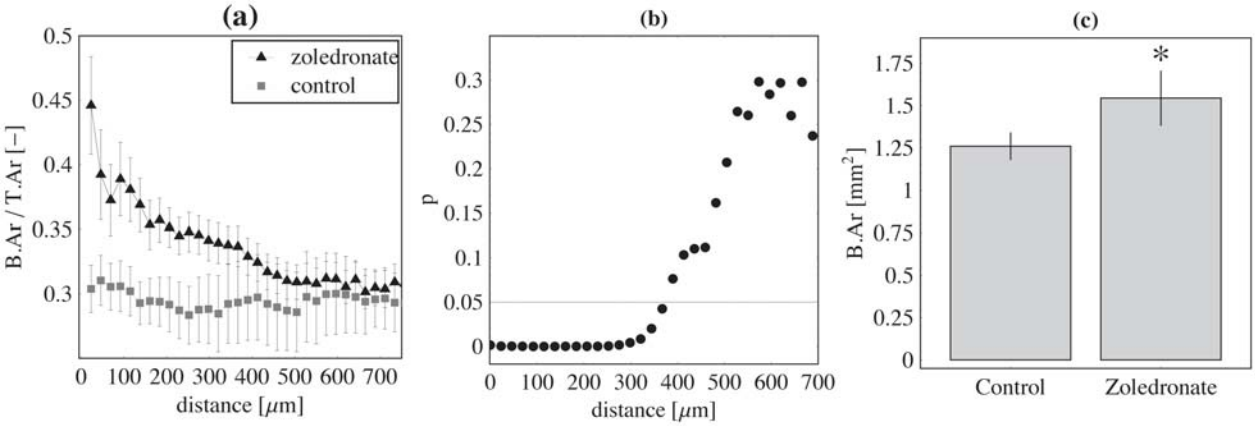


**Figure 4**: **(a)** Bone surface fraction (Mean±SEM) as a function of the distance from the implant coating. **(b)** Student *t*-test's *p*-value of B.Ar/T.Ar comparison between zoledronate-group and control-group as a function of the distance from the coating: the effect of local zoledronate is significant up to 400μm from the coating. **(c)** Total periprosthetic bone surface in a 400μm thick layer around the implant for zoledronate group and control group (Mean±SD, *: $p<0.05$, compared to control, SD were calculated from the number of slices per group which were accounted for as repetition of the density measurement of the same group).

observed: In non-osteoporotic rats, zoledronate coated implants induced an increase of 43% of B.Ar/T.Ar at the implant surface, while in osteoporotic rats the increase was 20% at 3 weeks post-surgery. In the present osteoporotic sheep model, at 4 weeks post-surgery, we observed an increase of 50%, which is significantly greater than the effect in rats. Moreover, the distance from the implant over which the effect of zoledronate is significant is five times greater in osteoporotic sheep than in osteoporotic rats.

The observed difference cannot be explained simply with this data. It can be related to prolongation of one week in the post-surgery delay, to an enhanced reaction of sheep bone cells to zoledronate, or to the initial difference in bone density. Despite the questions that remain unanswered, these results show that a small dose of zoledronate delivered locally in osteoporotic sheep bone efficiently increases periprosthetic bone density in a way similar to what was previously observed in rats.

This result is concordant with the very recent study of Goodship *et al*., in which intravenous administration of zoledronate pre- peri- and postsurgery reduced periprosthetic cortical osteopenia in a sheep model of hip replacement (Goodship *et al*., 2008). Therefore, zoledronate treatments seem to have a beneficial action on periprosthetic bone, cortical or trabecular.

The goal of local bisphosphonate release is to increase the implant fixation strength, but a complete pullout study would have required supplementary animals. Pullout force is mainly influenced by bone density in a thin layer of bone, extending 20μm radially from the implant (Peter *et al*., 2005). In osteoporotic rats, an increase of 20% of bone volume fraction in the 20μm layer induced nearly a 100% increase in pullout force (175±70N with 2.1μg compared to 90±30N for controls). However, the exact effect on pullout force in the sheep model cannot be calculated from rat models, as the scale of bone trabeculae and the bone density are very different in these species. But, with more than 2-fold increase in bone volume fraction in the 20μm layer, it is likely that an increase in pullout force would be observed as well in the present study.

Zoledronate, like other bisphosphonates, has been shown to limit bone loss in patients with osteoporosis (Glatt, 2001), while also inducing a beneficial impact on microarchitectural properties of trabecular bone (Poole *et al*., 2007; Recker *et al*., 2008). In the present study we did not assess other microarchitectural properties of periprosthetic bone than B.Ar/T.Ar. However, some of these properties, such as the bone volume fraction are mathematically linked to B.Ar/T.Ar (Parfitt *et al*., 1987; Revell, 1983).

**Clinical relevance of the study**

The clinical aim of coating orthopaedic implants with zoledronate is to improve the fixation of orthopaedic implants in patients with weak bone. Benefits of zoledronate local delivery were previously observed in osteoporotic rats. The present study further extended these results to a large animal model. The measured increase of

**Table 3**: Comparison of the effect of 2.1μg zoledronate / implant versus control on periprosthetic B.Ar/T.Ar in a 20μm layer, and distance of the effect from the implant. Rats and osteoporotic rats data adapted from (Peter *et al*., 2005) and (Peter *et al*., 2006), respectively.

| | B.Ar/T.Ar Control | B.Ar/T.Ar Zoledronate | Relative Increase | Distance of the effect |
|---|---|---|---|---|
| **Rats** | 0.48±0.04 | 0.69±0.03 | 43% | 250μm |
| **OVX rats** | 0.46±0.03 | 0.55±0.03 | 20% | 70μm |
| **OVX sheep** | 0.30±0.07 | 0.45±0.08 | 50% | 400μm |

periprosthetic bone density supported that local zoledronate delivery significantly improves the implant fixation in osteoporotic sheep.

To further validate the use of implants with local zoledronate delivery, the next set of experiments should be performed with full load bearing implants to quantify the combined effects of mechanical stimulus and zoledronate release to the response of periprosthetic bone.

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## Discussion with Reviewers

**J. Green:** In the absence of extensive clinical data, the relative practical benefits of local versus systemic bisphosphonate delivery are still debatable. Whilst local delivery from a coated implant may reduce the risk of side effects, a patient requiring an implant is likely to have osteoporosis at other sites and would thus benefit from systemic drug exposure. Moreover, after a surgical intervention to implant a prosthesis, the surrounding bone is highly active and shows enhanced bisphosphonate uptake, calling into question the need for local delivery from coated implants.

**Authors:** Some patients requiring orthopaedic implants are likely to have osteoporosis. Regarding these patients: in cases of undiagnosed and untreated disease, a zoledronate-coated implant would certainly offer benefits compared to uncoated implants, as indicated by the present pilot study. In cases of patients already treated with bisphosphonate, there is no experimental evidence that zoledronate-coating would provide a better fixation than a systemic administration. However, while zoledronate-coating provides an immediate release of a very small amount of drug at the desired location (which in this case is independent of the rate of activity of the surrounding bone), systemic treatment provides a continuous drug uptake on a long-term basis. Thus, the optimal implant fixation in patients with osteoporosis may be obtained by a combined approach of local and systemic treatment, rather than one or the other, taking advantages of both approaches. However, this hypothesis has yet to be demonstrated experimentally.

**D. Little:** This small paper suggests the possibility that local bisphosphonates can improve osseointegration of implants. Do authors have any estimate on the duration of such a positive effect?

**Authors:** Our objective was to obtain an increase of fixation at short-term. Indeed, clinical data suggests that a good short-term implant fixation is generally followed by a strong long-term fixation, when compared to implant with early loss of fixation. Based on these results, we believe that the benefit observed after 4 weeks should last for much longer. But, the duration of the beneficial effect depends on many factors such as the mechanical environment and the rate of elimination of the drug, thus the exact duration remains to be determined experimentally.

**R.G. Richards:** The reduced bone volume / trabecular volume is taken as an osteoporotic model of osteoporosis. Is this really a model of osteoporosis or osteopaenia? Do the authors think that any sheep model is actually a realistic model of osteoporosis or more of osteopaenia?

**Authors:** To be exact the presented data is a model of severe osteopaenia. There are actually no animal models that realistically reflect the human condition of osteoporosis. However, to our knowledge the OVX sheep model is the best model available to study orthopaedic applications in osteoporotic bone (see Turner, 2002).

**J. Green:** As bone loss in the ovine osteoporosis model can be influenced by breed, season, diet etc, the omission of any data to confirm the extent of the osteopaenia/ osteoporosis in these 3 animals is a concern. This could have been partially addressed by using each animal as its own control with a zoledronate coated implant in one femur and an uncoated control implant on the contra-lateral side – I doubt whether there would have been any systemic drug exposure from the coated implant.

**Authors:** Strictly speaking the condition of these animals is osteopaenia (based on BV/TV standard deviations) but the severe bone loss of 30% detected in iliac crests biopsies suggests that this model is somewhat comparable to osteoporosis condition in some human bones. Indeed, when planning the study, our concern was that the drug could influence the contra-lateral side bone density, which is why we did not use each animal as its own control. *A posteriori*, the extent of the effect of the drug is very limited (400µm) and thus there would certainly be no systemic effect. This supports the own-control design in the future.

**R.G. Richards:** Long term bisphosphonates have been seen to increase cortical bone thickness in elderly osteoporotic patients, yet recently it has been observed that this new cortical bone is very brittle and if fractured, very difficult to repair. I assume this is because the natural remodelling is knocked out by the bisphosphonates and the osteoclasts no longer function. What effect would local bisphosphonates have on the quality of the osseointegrated bone formed?

**Authors:** In the case of patients not exposed systemically to bisphosphonate in parallel to the presented technique, we do not think the quality of the bone is an issue on a long-term perspective: the coated dose is available in the bone only once and is slowly retrieved from the coating and from the surrounding bone with normal metabolic processes. Therefore the remodelling of the periprosthetic bone should be reactivated after some time, and the damaged bone renewed.