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Topical Bisphosphonate Augments Fixation of Bone-grafted Hydroxyapatite coated Implants, BMP-2 causes Resorption-based decrease in Bone

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Abstract

Bone allograft is used in total joint arthroplasties in order to enhance implant fixation. BMPs are known to stimulate new bone formation within allograft, but also known to accelerate graft resorption. Bisphosphonates are strong inhibitor of bone resorption. The aim of this study was to investigate whether the bisphosphonate zoledronate was able to counteract the accelerated graft resorption without interfering with the BMP induced bone formation. In the present study the two drugs alone and in combination were studied in our canine model of impaction bone grafting. We included 10 dogs in this study. Cancellous allograft bone grafts were soaked in either saline or zoledronate solution (0.005 mg/mL) and then vehicle or BMP2 (0.15 mg rhBMP2) was added. This produced four treatment groups: A) control B) BMP2 C) zoledronate and D) BMP2+ zoledronate. The allograft treated with A,B,C or D was impacted into a circumferential defect of 2.5 mm around HA-coated porous Ti implants. Each dog received all four treatment groups with two implants in the distal part of each femur. The group with allograft soaked in zoledronate (C) showed better biomechanical fixation than all other groups ($p < 0.05$). It had less allograft resorption compared to all other groups ($p < 0.005$) without any statistically significant change in new bone formation. The addition of BMP2 to the allograft did not increase new bone formation significantly, but did accelerate allograft resorption. This was also the case where the allograft was treated with BMP2 and zoledronate in combination (D). This caused a decrease in mechanical

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implant fixation in both these groups compared to the control group, however only statistically significant for the BMP2 group compared to control. The study shows that topical zoledronate can be a valuable tool for augmenting bone grafts when administered optimally. The use of BMP2 in bone grafting procedures seems associated with a high risk of bone resorption and mechanical weakening.

Keywords

implant fixation; allograft; bisphosphonate; bone morphogenetic protein; joint replacement

1. Introduction

Failed joint replacements are often complicated by osteopenic and insufficient host bone. At surgical revision, the goal is to achieve stable early fixation of the implant components, as this is predictive for the long-term survival of the joint replacement^{1,2}. One well-established way of managing this is with the use of impacted allograft bone. The bone graft provides mechanical support of the implant and a scaffold for new bone ingrowth. Healing of such grafted defects is, however, inconsistent and often the bone grafts resorb or remain encapsulated in fibrous tissue around the implant instead of being replaced by the patient's own bone³.

Recombinant human BMP2 is a bone anabolic substance that stimulates differentiation of osteoblasts. When delivered in a collagen sponge, it is FDA-approved as an adjuvant therapy for augmenting lumbar spinal fusion and healing of tibia shaft fractures. The clinical use of BMPs with allograft bone has given divergent results⁴⁻⁶. These clinical results indicate that addition of BMPs to allograft might enhance healing of tibia shaft fractures but fail to show effects on fixation of total joint replacements. One potential problem with adding BMPs to allograft is accelerated graft resorption. Experimental data suggests that adding BMPs to allograft is not only associated with increased formation of new bone, but also with increased resorption of the allograft^{4,7}. Such accelerated allograft resorption has been thought to cause an early intermittent period of weakened implant fixation, pending remodeling of the immature woven bone.

N-bisphosphonates induce cell apoptosis by attaching to exposed bone mineral leading to it being resorbed by osteoclasts⁸. In terms of bone metabolism, they are anti-catabolic and slow down resorption of bone. There is conflicting evidence as to the effect of bisphosphonates on new bone formation. Some studies suggest that bisphosphonates can initiate osteoblastic differentiation and upregulate BMP2 gene expression^{9,10}. Other studies find increased new bone formation with topically administered bisphosphonates, but attribute this to increased osteoconductive area and scaffolding capacity due to the preservation of existing bone^{11,12}.

It would be rational to combine a bisphosphonate with rhBMP2 in order to reduce the increased bone resorption, but still benefit from the BMP-induced formation of new bone. In a previous animal experiment we looked at the effect of the bisphosphonate pamidronate and rhBMP2 on allografted implant fixation – alone and in combination – we found that

rhBMP2 stimulated formation of new bone around the implant, but also lead to an accelerated resorption of the bone graft¹³. Pamidronate alone preserved the allograft bone but prevented any new bone formation. When the two substances were combined, there was preservation of the allograft but still no new bone formation within the grafted gap. We postulated this may have been caused by delivering too high a dosage of the pamidronate, leading to a presence of unbound pamidronate within the bone-grafted gap around the implant. A later study showed a beneficial effect of zoledronate on bone-grafted implant fixation, when used in small dosages and where excess zoledronate was removed by rinsing¹².

Other experimental studies using a bone conduction chamber in a rodent bone have found that combination of local or systemic bisphosphonate and BMP-7 increases new bone formation while preserving the allograft in the bone chamber^{14,15}. None of the studies tested osseointegration of implants.

The object of the current experiment was to investigate whether combining a specific anabolic substance with a specific anti-catabolic drug, at dosages derived from previous studies, could improve implant fixation and osseointegration of the graft bed as well as the implant. In combining the two substances, we anticipated a balanced outcome, where new bone formation would be stimulated but without the adverse effect of concomitant bone resorption. We hypothesized, that the combination of rhBMP2 and zoledronate could increase fixation and osseointegration of porous Ti and HA-coated implants inserted into impacted, morselized allograft bone. For experimental purposes, we defined this as increasing mechanical fixation, increasing new bone formation, reducing the presence of fibrous tissue and preservation of the allograft.

2. Materials and Methods

2.1 Experimental design (Table 1)

Ten dogs were included and each animal received four implants. The implants were placed into the distal part of the femur with two implants in each femur (Figure 1). The implants were surrounded by a 2.5 mm circumferential defect, which was filled with impacted, morselized allograft added rhBMP2 and/or zoledronate. The four-armed allograft intervention was conducted in a paired design, where each dog received all four treatments. The allograft groups were A) control, B) BMP2, C) zoledronate and D) BMP2 + zoledronate (Table 1). The implantation site of each group was alternated systematically with random start. The observation time was 4 weeks.

2.2 Graft material

The bone graft was harvested immediately post mortem under sterile conditions from two dogs not included in the study and stored at -80° . The proximal humerus and the distal femurs were used. Prior to surgery, the bone graft was thawed and prepared: all soft tissue and cartilage was removed, and the bone was morselized with a standard bone mill (Biomet®, Warsaw, Indiana, USA) on fine setting, creating bone chips of 1 to 4 mm size. The chips from the different bones were mixed together. Bone chips longer than

approximately two mm were sorted out, and the allograft divided into four tightly compressed portions of 1 mL in standardized sterile containers. The portions were weighed (mean weight 1.16 grams, range 1.15 – 1.17) and stored at –80°C. The recombinant human BMP2 was purchased as Infuse (Medtronic Sofamor Danek USA Inc., Memphis, TN, USA) and applied as reconstituted protein 1.5 mg/mL solvent but without the bovine collagen carrier of the device. The zoledronate was purchased as Zometa 4 mg / 5 mL (Novartis Europharm Ltd. Horsham, West Sussex, England) for intravenous infusion.

At surgery, the 1 mL portions of allograft bone was rinsed in 20 mL saline for 1 minute three times. In the control group, the allograft was otherwise left untreated. In the BMP-group, 0.1 mL of 1.5 mg/mL solvent was added to the allograft, corresponding to 0.15 mg rhBMP2. In the BP-group, the allograft was soaked for three minutes in 1 mL zoledronate 0.005 mg/mL. The allograft was drained and then rinsed in 20 mL saline for 1 minute three times to remove unbound zoledronate. In the combined BMP/BP-group, the allograft was prepared as described for the BP-group, and then 0.15 mg rhBMP2 was added.

2.3 Implants

We used 40 hydroxyapatite-coated porous titanium alloy (Ti-6Al-4V) implants for the experiment, manufactured by DePuy Inc. (Warsaw, IN, USA). The porous surface was achieved with spherical commercially pure Ti beads giving a porosity of 40-50% and an average pore size of 250-300 µm. All Ti-6Al-4V substrates were in accordance with ASTM standard F-136. All Titanium beads were in accordance with ASTM standard F-67. All beads were attached by a sintering process with heat and pressure handling in vacuum furnace. After passivation, the surface was coated with hydroxyapatite by plasma spray technique, giving a coating thickness of 40-60 µm, HA crystallinity of 78.4 wt %, Ca/P ratio 1.67 (Bio-Coat, Detroit, MI, USA). The final implants were cylindrical with a height of 10 mm and nominal 6 mm diameter. A footplate of 11 mm diameter was attached on one end of each implant. When inserted into an 11 mm drill hole, this centered the implant and provided a uniform 2.5 mm defect around it. Only the cylindrical part of the implant was HA-coated (Figure 1). After grafting the defect, an 11 mm diameter top-washer was mounted on the outer end of the implant to secure stability, concentricity and containment of the graft material.

2.4 Animals

Ten skeletally mature female American hounds with a mean weight of 25.1 kg (range, 23.5-26.5 kg) and age 13-15 months were included into the study. Two additional dogs served as bone graft donors. The dogs were bred for scientific purposes, all procedures in this study were approved by the Animal Care and Use Committee of the Minneapolis Medical Research Foundation.

2.5 Surgical procedure

Under general anesthesia and with sterile conditions, the femoral epicondyles were exposed, starting with a medial incision. The joint capsule was opened, identifying the collateral ligament. A 2.5 mm guide wire was placed perpendicular to the epicondylar surface, 18 mm from the distal edge of the condyle and 14 mm from the anterior edge of the condyle. A

cannulated drill bit of 11 mm diameter was then used to create a 12 mm deep drill hole. A drill speed of 2 rotations per second was used to avoid thermal damage to the bone. The implants with footplates were inserted with a specially designed impaction tool to secure uniform central placement. The same tool was then used to impact 1 mL bone graft corresponding to the four different treatment groups into the peri-implant gap/defect. Finally, the top washer was mounted to secure graft containment and implant concentricity, and the soft tissues closed in layers. The procedure was repeated for the lateral side, and then for the contralateral femur.

At the last day of surgery, two canine cadaver femora underwent the same procedure with grafted placement of four implants. These implants were used to represent time-zero status for the allograft.

All implants were inserted by the same operating surgeon (JB). The dogs were given Ceftriaxone 1 g administered immediately before each surgery and for 3 days postoperatively. Buprenorphine hydrochloride 0.3 mg/mL 0.0075 mg/kg/day i.m. was given as postoperative analgesic treatment. The dogs were allowed unrestricted weight bearing immediately after both surgeries. After four weeks observation time, the dogs were sedated and euthanized with an overdose of hypersaturated barbiturate.

2.6 Specimen preparation

The distal part of the femur was removed and stored frozen at -20°C immediately after retrieval. The outermost 0.5 mm of the implant-bone specimen was cut off and discarded. The rest of the implant with surrounding bone was divided into two sections perpendicular to the long axis of the implant with a water-cooled diamond band saw (Exact Apparatebau, Nordenstedt, Germany). Cutting of the bone-implant specimen was done with a velocity of approximately 0.5 mm pr. second without affecting the stability of the implant in the bone. The outermost section was cut to a thickness of 3.5 mm and stored at -20°C until mechanical testing. The innermost section was cut to a thickness of 5.5 mm and prepared for histomorphometry (Fig. 2). These specimens were dehydrated in graded ethanol (70-100%) containing basic fuchsin, and embedded in methylmethacrylate (MMA, Merck, Hochenbruun, Germany). Using vertical sectioning technique^{16,17}, each specimen was cut into four 30 μm thick histological sections with a microtome (KDG-95, MeProTech, Heerhugowaard, Holland). Finally, these were surface counterstained with 2% light green for 2 minutes, rinsed and mounted on glass. This preparation provided red staining of non-calcified tissue and green staining of calcified tissue. The different types of calcified tissues such as woven bone and lamellar bone were discriminated based on their morphological characteristics¹⁸.

2.7 Mechanical testing

Thawed specimens were tested to failure by axial push-out test on an MTS Bionics Test Machine (MTS, Eden Prairie, MN, USA) using a 2.5 kN load cell. Testing was performed blinded and in one session. The transversely cut specimens were placed with the cortical side facing up on a metal support jig with the implant ($\varnothing$ 6 mm) centered over a 7.4 mm opening and under a cylindrical test probe of 5 mm diameter. A preload of 2 N defined the

contact position for the start of the test. The implants were then pushed out of the surrounding tissue in the direction of the implant axis at a displacement rate of 5 mm/min. Load versus implant displacement data were continuously recorded. All mechanical parameters were normalized by dividing the force with the cylindrical surface area of the implant section tested. Maximum shear strength (Strength; Pa) was determined from the maximum force applied until failure of the bone-implant interface. Maximum shear stiffness (Stiffness; Pa/mm) was obtained from the slope of the linear section of the load versus displacement curve. Total energy absorption (Energy; J/m²) was calculated as the area under the load displacement curve until failure.

2.8 Histological evaluation

Blinded quantitative histomorphometry was performed using the stereological software newCAST (Visiopharm A/S, Horsholm, Denmark). The used stereological method has previously been described in detail¹⁹. With the aid of the software, two regions of interest were defined: Zone 1 from the innermost parts of the implant surface and 500 µm into the grafted defect, and Zone 2 in the 500-2000 µm part of the grafted defect. In Zone 1 the area fractions of new bone, bone graft, fibrous tissue, and marrow space covering the implant surface were quantified by line-interception technique¹⁶. In Zone 2 volume fractions of the same tissues in the grafted gap around the implant were quantified by point-counting technique²⁰. Bone was surface-stained green, and therefore easy to distinguish from the other tissues. Newly formed bone was woven, appearing less organized with large, round osteocyte lacunae. Lamellar bone was defined by its highly organized lamellas and lamella-oriented long, oval cell lacunae. Bone graft was lamellar bone and had empty osteocytic lacunae. Fibrous tissue was identified by its presence of clearly visible fibril fiber complexes and low cell density. Marrow space consisted of fat vacuoles and surrounding blood cells.

2.9 Statistical analysis

The mechanical data followed a normal distribution and fulfilled the assumptions for parametric evaluation with repeated measures ANOVA followed by paired t-test. The histological datasets were evaluated non-parametrically, as normal distribution could not be assumed for all parameters, in most cases because of values close to zero. These datasets were evaluated with Friedman repeated measures analysis of variance by ranks followed by Wilcoxon signed-rank test. For all datasets, differences between means and medians were considered statistically significant for p-values <0.05. Statistical analysis was performed using Stata/IC 11 software (StataCorp LP, College Station, TX, USA).

3. Results

3.1 Observations on animals

All ten dogs were fully weight bearing within three days after surgery and completed the four week observation period without signs of infection or other complications.

3.2 Mechanical tests (Tables 2a and 2b)

The zoledronate-treated BP group had the best mechanical implant fixation, whereas the groups where rhBMP2 alone and in combination with zoledronate were added to the

allograft had the poorest fixation. The zoledronate group had a 40% better fixation than the untreated control group by strength to failure ($p=0.011$), and a 55% better fixation measured on shear stiffness ($p=0.005$). The BMP2 and BMP2 + zoledronate groups had a decreased implant fixation in comparison to the control group measured on strength and stiffness, however only statistically significant for the BMP2 group ($p=0.021$ and $p<0.001$, respectively). There was no statistically significant difference between any of the four groups measured on the parameter total energy absorption before failure.

3.3 Histological observations (Figure 3, Tables 3a and 3b)

On the implant surface (Zone 1), we found that the implants in the zoledronate-treated group and the control group had 1/3 of the implant surface in direct contact with newly formed bone. In the zoledronate and BMP2 + zoledronate treated groups, only 22% of the implant surface was in direct contact with newly formed bone. This corresponds to a relative decrease of 50% compared to the control group, however only statistically significant for the BMP2 + zoledronate group ($p=0.009$). There was virtually no fibrous tissue or allograft bone in direct contact to the implants in any groups, hence no differences were observed.

In the grafted defect (Zone 2) allograft bone constituted 7.3% of the volume in the control group. In comparison, the allograft volume fraction was 25% in the zoledronate group ($p=0.005$), 0% in the BMP2 group ($p=0.006$) and 5.3% in the BMP2 + zoledronate group ($p=0.969$). New bone was formed in the grafted gap in all groups, and roughly 25% of the gap was occupied by newly formed bone. Virtually no fibrous tissue was present in the gap. There were no statistically significant differences between the groups in terms of new bone or fibrous tissue.

The four time-zero implants had a median volume fraction of allograft bone in the gap (Zone 2) of 41% (range 39-43%), which was higher than all treatment groups after four weeks observation time ($p<0.001$).

4. Discussion

The purpose of this study was to balance the increased bone turnover of BMP2 with an antiresorptive bisphosphonate to achieve a net increase of bone formation around grafted implants without excessive allograft resorption. The bisphosphonate zoledronate and rhBMP2 – alone and in combination – was added to morselized allograft bone. The bone graft was packed around HA-coated porous Ti implants.

We found that adding zoledronate to the bone graft improved implant fixation, but the addition of BMP2 – alone and in combination with zoledronate – decreased implant fixation. Implant osseointegration was decreased in both groups with BMP2. Whereas there was no difference between the four treatment groups in new bone formation within the grafted gap, the allograft underwent less resorption in the zoledronate group and resorbed completely in the BMP2 group. By combining BMP2 with zoledronate some of this excessive resorption was prevented, but only to the level of the control group and with no benefit in terms of increased mechanical implant fixation.

The canine model for grafted implants is well-established²¹, but like all experimental animal models has strengths and limitations. It models early implant fixation and osseointegration of uncemented implants inserted into a bed of impacted, morselized allograft bone. The surgical model is well-standardized and has a high degree of control of variables control. However, this control results in a trade-off that clinically important influences on implant fixation such as direct axial load and oscillating joint fluid pressure are not present. The surgeries are conducted in young, healthy canines and not in humans with osteopenic bone, which is often the case in revision arthroplasty. However the canine trabecular network is similar in trabecular thickness, connectivity and spacing to human cancellous bone²². The long-term effect of the adjuncts used in this experiment have not been investigated, and the present study is only designed to draw conclusions on its effects on early-phase implant fixation. The model allows paired comparison of four bone-grafted implants inserted into the same animal, by which variance due to inter-individual biological differences is reduced. In this study, we used HA-coated implants. The HA-coating improve osseointegration of the implant at the bone-to-implant interface, but has little effect further away from the implant²³. Furthermore, in contrast in titanium, zoledronate has a strong affinity for HA. It is not possible from this study to conclude on the effect of titanium coated in the same model.

Both treatments were administered topically. This secured presence of the drugs in the graft bed, which is initially avascular, and facilitated uniform treatment of the bone graft. Clinically, topical administration has the advantage of eliminating poor patient compliance and the risk of systemic side effects that can occur with systemic administration. Furthermore, there is variability in delivery, as the time point of vascularization is unknown. Systemic administration of the zoledronate, however, could potentially protect newly formed bone around the implant from later resorption, and increase the net amount of newly formed bone, as proposed by Aastrand²⁴.

Infuse consists 12 mg rhBMP2 in a bovine collagen carrier, however here we used the BMP2 without the bovine collagen carrier as done previously in a similar model with OP-1²⁵. Because of the constrained nature of the defect in our model we chose a relatively lower dose (0.45 mg 1.5 mg/mL BMP2 per 1 mL allograft) corresponding to two standard doses rhBMP2 (two Infuse devices) per femoral head graft. Previous studies have shown that one standard dose OP-1 per human femoral head graft in a rabbit implant model had no effect on bone remodeling²⁶ whereas four standard doses OP-1 per human femoral head graft in an implant model in dogs gave excessive remodeling²⁷. Based on a previous study in the same model, we expected this dose BMP2 to provide a stimulatory effect on new bone formation as well as allograft resorption¹³. This would cause a negative net effect on implant fixation at this early observational time point, where the mechanically stable impacted allograft was resorbed and the implant was supported by newly formed immature woven bone alone¹³. We chose to keep the BMP2 dose unchanged, as it seemed important to deliver an adequate anabolic response for the anti-catabolic zoledronate treatment to prove effective²⁸. Furthermore, we chose to use the BMP2 without the bovine collagen carrier, as done in our previous studies, although little is known about release kinetics of BMP2 from the allograft.

Osteoclasts are selectively subject to bound zoledronate due to the targeting of bisphosphonates to hydroxyapatite and the ability of osteoclasts to release the substance by acidification. The effect of bisphosphonates on other cells is less clear, but its ability to penetrate the cell wall without an active process such as pinocytosis is very limited due to the bulkiness and negative charge of the phosphonate group²⁹. The administration of zoledronate in this study was based on a study in the same model where the bone graft was rinsed free of unbound bisphosphonate after soaking in three different concentrations, showing a dose-response relationship and a beneficial effect on allograft protection as well as new bone formation (1 mL zoledronate 0.005 mg/mL)¹².

In the present study, the addition of zoledronate alone to the bone graft decreased the resorption compared to the control group. Some resorption did occur in the zoledronate group, as the volume fraction of allograft in the gap was reduced from 40% at surgery to 25% after four weeks. This indicates that the protective effect is relative, and that it is probably reduced with time. The bisphosphonate molecules attach to bone until they are released by the very osteoclasts in which they induce apoptosis. Eventually, the surface of the bone graft will be unprotected, after which the resorption can occur at a natural speed. It is beyond the scope of the current experiment to calculate the timing of these events or even detect an increase of resorption with time. However, the results suggest that this process is delayed by a protective layer of bisphosphonate on the bone graft.

The zoledronate administration method used here caused no inhibition of new bone formation. Quite the contrary; there was a tendency towards more new bone in the grafted gap, and the implant osseointegration was significantly better than in the BMP2-treated groups. This would be consistent with that the anabolic effect observed in the zoledronate group was due to an increase in the bone graft surface area, providing a larger osteoconductive area to scaffold new bone ingrowth. New bone formation could perhaps be augmented further by the addition of a bone anabolic agent, but the opposite was the case in this experiment. New bone formation - and in particular implant osseointegration - is essential for implant anchorage, and it seems clear that the presence of both graft and new bone are prerequisites for good early implant fixation.

BMP2 caused a complete resorption of the allograft at four weeks. Furthermore, there was only a slight increase in new bone formation around the implant, and this was not statistically significant. This caused a negative net effect on implant fixation at this early observational time point, where the mechanically stable impacted allograft was resorbed and the implant was supported by newly formed immature woven bone alone, as shown previously with OP-1²⁷ and BMP2¹³. When zoledronate was combined with BMP2, the bone graft was protected from resorption to some degree, and the amount of allograft around the implant was comparable to the control group. There was no detectable advantage in terms of increased new bone formation, and implant fixation was comparable to the control group. Most likely, the employed dose was too high, even with an adequate administration of zoledronate. Whether a lower dose BMP2 in combination with the employed dose zoledronate could augment fixation of grafted implants even more remains unanswered.

5. Conclusion

We were able to change the balance point between bone formation and resorption by adding zoledronate to the allograft around bone grafted implants. We were unable to further change this by adding BMP2, perhaps due to a too large dose. Both zoledronate and BMP2 have been shown to be powerful agents in influencing periprosthetic bone metabolism, and this study clearly demonstrates that topical zoledronate can augment periprosthetic bone healing to achieve improved mechanical fixation of the grafted implant. However; BMP2 seems to elevate bone turnover rather than selectively stimulating new bone formation. Both substances have the potential to harm implant fixation rather than augment it, and the therapeutic window of the agents in a human setting is still unknown.

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Highlights

- BMP2 increased allograft resorption and decreased implant fixation
- Zoledronate preserved allograft and increased implant fixation
- Zoledronate was not able to counteract the increased graft resorption induced by BMP2

Figure 1.

A diagram illustrating the four implants inserted into distal part of femur. All implants were surrounded by a 2.5 mm circumferential gap packed with morselized allograft added (A) nothing (control) (B) BMP2 (C) zoledronate and (D) BMP2+ zoledronate

Figure 2.

A schematic diagram shows the specimen preparation. Each bone-implant specimen is cut into two pieces: 3.5 mm specimen (A) for mechanical push-out test, and a 5.5 mm specimen (B) for histomorphometrical analyses. Before cutting specimen A and B the superficial endcap is removed and the outermost 0.5 mm of the bone-implant specimen is cut-off and discarded. The transversely cut specimens (A) were placed with the cortical side facing up on a metal support jig with the implant ($\varnothing$ 6 mm) centered over a 7.4 mm opening and under a cylindrical test probe of 5 mm diameter. The 5.5 mm (B) is randomly rotated around the long axis of the implant after which four parallel sections are cut parallel to the long axis of the implant

Figure 3.

Representative photomicrographs of samples from the same animal. The samples were stained with basic fuchsin and counter-stained with 2% light green. Implant appears as black, marrow as red, and bone as green. Note the preserved allograft and new bone formation in the zoledronate group.

Table 1

Experimental design

Intervention groups (Tot. no. dogs=10)	Graft material	Treatment
CONTROL (n=10)	Allograft 1 cm ³	-
BMP2 (n=10)	Allograft 1 cm ³	BMP2
ZOL (n=10)	Allograft 1 cm ³	zoledronate
BMP2 + ZOL (n=10)	Allograft 1 cm ³	BMP2 + zoledronate

Each dog received 4 porous-coated Ti implants grafted with 1 cm³ allograft w/wo treatments in the distal part of femur each dog. To counteract possible systematic site-dependent differences, the different treatments were rotated systematically with random start within the four implantation sites.

Table 2a

Mechanical pushout test [mean, sd]

	Ultimate Shear Strength (MPa)		Apparent Shear Stiffness (MPa/mm)		Total Energy Absorption (kJ/m ²)	
CONTROL	5.4	(2.2)	22.3	(8.0)	1.22	(0.53)
BMP	4.2	(1.3)	15.2	(6.1)	1.09	(0.55)
ZOL	7.6	(2.5)	34.5	(6.1)	1.26	(0.53)
BMP + ZOL	5.1	(2.0)	21.0	(9.6)	1.28	(0.58)
ANOVA	p<0.001		p<0.001		p=0.794	

Table 2b

P-values for paired comparisons of mechanical parameters between allograft treatment groups in Table 2a. P-values below 0.05 were considered statistically significant and are marked **bold**.

p-value	Ultimate Shear Strength			Apparent Shear Stiffness			Total Energy Absorption		
	BMP	ZOL	BMP + ZOL	BMP	ZOL	BMP + ZOL	BMP	ZOL	BMP + ZOL
CONTROL	0.021	0.011	0.747	<0.001	0.005	0.666	-	-	-
BMP		0.001	0.098		<0.001	0.036		-	-
ZOL			0.021			0.008			-
BMP + ZOL									
	(paired t-test)			(paired t-test)			(no test performed)		

Table 3a

Histomorphometrical data (tissue fractions in %)

Histomorphometry data are presented as medians (interquartile range)

Implant Surface Ongrowth: Tissue area fraction in %						
	New Bone		Allograft		Fibrous Tissue	
CONTROL	32	(23-32)	0.0	(0.0-0.2)	0.1	(0.0-0.8)
BMP	22	(12-29)	0.0	(0.0-0.0)	0.0	(0.0-0.0)
ZOL	33	(26-39)	0.0	(0.0-0.1)	0.0	(0.0-0.3)
BMP + ZOL	22	(15-26)	0.0	(0.0-0.0)	0.0	(0.0-0.0)
ANOVA	p=0.040		p=0.176		p=0.146	

Gap Ingrowth: Tissue volume fraction in %						
	New Bone		Allograft		Fibrous Tissue	
CONTROL	21	(18-25)	7.3	(4.2-10.4)	0.0	(0.0-0.0)
BMP	25	(20-32)	0.0	(0.0-0.4)	0.0	(0.0-0.0)
ZOL	26	(21-33)	25	(23-29)	0.0	(0.0-0.5)
BMP + ZOL	27	(25-29)	5.3	(4.3-8.8)	0.0	(0.0-0.0)
ANOVA	p=0.171		p<0.001		p=0.100	

Table 3b

P-values for paired comparisons of histomorphometrical parameters between allograft treatment groups in Table 2a. P-values below 0.05 were considered statistically significant and are marked **bold**.

p-value	Implant Surface Ongrowth - New Bone			Gap Ingrowth Allograft		
	BMP	BP	BMP+B P	BMP	BP	BMP+B P
CONTROL	0.075	0.333	0.009	0.006	0.005	0.959
BMP		0.047	0.799		0.005	0.005
ZOL			0.022			0.005
BMP + ZOL						