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Does tobramycin impregnation of allograft bone affect implant fixation? – An experimental study in 12 dogs

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Abstract

Clinical trials have used antibiotic impregnated impacted bone allograft in revisions of infected arthroplasties. By this method high local antibiotic concentration and good control of infection was achieved. Toxicity studies, however, suggest that high local antibiotic concentration can impair osteoblast replication. We therefore asked whether impregnating morselized allograft bone with different quantities of tobramycin before impaction would impair implant fixation. We implanted three cylindrical (10 mm × 6 mm) porous-coated titanium implants into the distal femurs of 12 dogs. The implants were surrounded by a circumferential gap of 2.5 mm into which a standardized volume of morselized allograft bone, with or without tobramycin, was impacted. In each animal, the bone graft was impregnated with either 0 mg (control), 50 mg (low dose), or 200 mg (high dose) of tobramycin per 1 mL of bone graft. At the end of the 4 weeks experimental period, the implants with surrounding bone were evaluated by histomorphometric analysis and mechanical push-out test. We found no difference between the treatment groups regarding new bone formation, bone graft resorption, or implant fixation. There was, however, a tendency toward a decrease in implant fixation with higher tobramycin dose. The present study is unable to provide evidence on whether the use of topical tobramycin with allograft is safe or whether it indeed can impair implant fixation. The tendency toward an impaired implant fixation warrants further preclinical studies. Its current clinical use should be weighed against its possible positive effects on preventing infection in complicated revisions.

Keywords

morselized allograft bone; tobramycin; impregnation; osseointegration; implant fixation

INTRODUCTION

Loosening of an artificial joint prosthesis is a painful and debilitating condition that can be treated only by reoperation. This procedure accounts for approximately 15% of all hip

replacement operations performed in Denmark between 1995 and 2010.¹ Osteolysis and bone loss, which are often seen in revision surgery, are great challenges for the surgeon. Bone stock deficiency may be remedied by means of impacted morselized allograft bone, which is a well-established method for obtaining additional arthroplasty support.^{2–5}

Systemically administered antibiotics are widely used as prophylaxis against infection. Since the impacted bone graft is initially an avascular area, systemic antibiotics cannot easily gain access to the graft site whereby the risk of postoperative infection is heightened.⁶ Local antibiotic delivery has, therefore, become a much-researched area in recent years. *In vitro* studies have shown morselized bone grafts to have the ability to act as delivery vehicles of antibiotics.^{7–10} The release profile of tobramycin impregnated bone graft has demonstrated a steady release with a tobramycin concentration in the surrounding liquid above minimal inhibitory concentration for the common pathogens in orthopedics throughout a test period of 28 days.¹¹ Local administration makes it possible to achieve a local drug concentration much higher than the therapeutic level without reaching a toxic serum concentration.¹² Toxicity studies have, however, shown that a high local antibiotic concentration can impair osteoblast replication.^{12,13} Despite this concern, small clinical trials using tobramycin or vancomycin-impregnated bone grafts in revisions of infected arthroplasties have been conducted.^{6,14,15}

Tobramycin is a widely used aminoglycoside.⁸ It is a bactericidal antibiotic, effective against many Gram-negative and Gram-positive pathogens, and covers the majority of pathogens encountered in orthopaedic surgery.^{11,16} It also has a low resistance rate and low allergy rate.¹⁶ A high tobramycin concentration has shown a toxic effect on osteoblast replication, where a local concentration of tobramycin above 400 µg/mL yielded a decrease in osteoblast replication, and a concentration of 10,000 µg/mL or above caused cell death.¹²

We therefore asked whether impregnating morselized allograft bone with different quantities of tobramycin before impaction would impair implant fixation as evaluated by histomorphometry and mechanical push-out test.

MATERIALS AND METHODS

The experiment was designed as a paired, randomized, controlled animal study in 12 female American hounds with a mean weight of 24 kg (22–26 kg) and a mean age of 16 months (12–21 months). Prior to conducting the experiment, a sample size calculation was performed. Given the standardized conditions in the model, a detection level of relevant group differences was set to 30% relative change in the biomechanical parameters (strength, stiffness, energy) and histomorphometrical parameters (new bone, allograft, fibrous tissue ingrowth, and ongrowth, respectively). The two-sided α was set to 5% and the β set to 20%. Two extra animals were added to the calculated sample size of 10 to counteract a decrease in power if implants from one or two animals were lost to follow-up and subsequent analysis. It is the authors' experience from previous experiments with this model, that this detection limit of 30% is appropriate for later clinical manifestation. Each animal received a total of three porous-coated cylindrical titanium implants in the distal femurs, one control and two intervention implants. Each implant was surrounded by a peri-implant gap of 2.5 mm

(Figure 1). Using an impaction technique; we filled the gap around each implant with morselized allograft bone with or without tobramycin. The three implants in each animal were implanted and grafted in the following order: (1) allograft, (2) allograft + low dose tobramycin, and (3) allograft + high dose tobramycin. In order to minimize antibiotic leakage between treatment groups, the two intervention implants were inserted into the same femur while the control implant was inserted into the opposite femur. The implantation site (left/right limb and medial/lateral epicondyle) of each treatment group was alternated systematically with a random start. The observation period was 4 weeks. The animals were raised for scientific purposes and housed individually but socialized in groups with 2 hours of daily exercise. The experiment was approved and monitored by the Institutional Animal Care and Use Committee (Minneapolis Medical Research Foundation, Minneapolis, MN, submission ID 08-08-02, protocol number 05-01). The surgeries and observations were performed at the Animal Care facilities of the Minneapolis Medical Research Foundation at the Hennepin County Medical Center (Minneapolis, MN), according to the regulations of the National Institute of Health. Preparation of the bone samples, mechanical testing, and histomorphometry were carried out at the Orthopaedic Research Laboratory of Aarhus University Hospital (Aarhus, Denmark). Each author certifies that his or her institution approved the animal protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

The animals were part of a multistudy series, and two additional unrelated studies were performed in the same test animals under the same anesthesia. In the humerus, a study evaluating the effect on implant fixation when adding local Simvastatin (1.0 mg) to a poly-DL-lactide surface coating was conducted. In the tibia a poly(methyl methacrylate) bone cement study was performed by testing the effect of topical bisphosphonate treatment of the bone cement interface. A drilled bone defect was filled with a zoledronate (Zometa, Novartis, Basel, Switzerland) solution of 0.05 mg/mL for 1 minute. This was followed by a 1-minute soak in saline to wash away any excess unbound Zolendronate. To minimize the risk of contamination between study groups, each implant was inserted through separate skin incisions with separate instruments. The bisphosphonate study in the tibia was conducted as the last surgery after all femur implants were inserted and the skin closed in layers.

For the experiment, we used 36 porous-coated titanium alloy (Ti-6Al-4V) implants (the surface coating was performed and donated by DePuy Inc, Warsaw, IN). The implants were cylindrical, with a mean diameter of 5.94 mm (SD, 0.09), and a height of 10 mm. Before autoclave sterilization, the implants were cleaned in an ultrasonic bath of trichloroethylene with final baths in alcohol. An 11-mm diameter bottom screw was threaded into each implant (Figure 1). When inserted into an 11-mm drill hole, the 11-mm bottom screw centered the implant, providing a uniform 2.5-mm peri-implant gap. After impaction of the variously prepared allograft, the gap was closed with an 11-mm top screw whose surface aligned with the bone cortex (Figure 2). This construct ensured implant stability and bone-graft containment

Bone allograft was harvested immediately post mortem and under sterile conditions from two dogs that were not included in the study. The proximal humerus, the proximal tibia, and the distal femur were used. After removal of all soft tissues and cartilage, the remaining

bone was morselized using a standard bone mill (Biomet Inc®, Warsaw, IN) on fine setting, which created bone chips of 1 mm to 3 mm. Bone chips from the two animals were mixed together and divided into 36 tightly compressed portions of 1 mL and stored frozen in sterile containers at -80°C.

Tobramycin powder was dissolved in sterile water at two concentrations: 200 mg/mL (low dose) and 800 mg/mL (high dose). At surgery, three portions of 1 mL-bone graft were thawed for 10 minutes at room temperature and evenly spread out in the bottom of separate small sterile metal containers. To each portion, we drop-wise added the following: Control group, 0.25 mL of sterile water; Low dose group, 0.25 mL of 200 mg/mL tobramycin solution (50 mg tobramycin/mL bone graft); High dose group, 0.25 mL of 800 mg/mL tobramycin solution (200 mg Tobramycin/mL bone graft). The bone graft absorbed all liquid and was stirred to ensure homogeneous distribution. The low dose level was comparable with concentration used in clinical trials and considered as a clinically relevant dose.¹⁵ The high dose group was included to reflect the upper boundary of a clinically relevant concentration, increasing the detectability of potential adverse effects. The mixtures were stored at room temperature for a minimum of 5 minutes before implantation.

A 1 g dose of ceftriaxone was given intravenously to each animal before surgery. The surgery was conducted under sterile conditions with the animal under general anesthesia induced with 5% thiopental before intubation and maintained with 1.5% isoflurane. Buprenorphine hydrochloride was used for analgesia. The femoral epicondyles were exposed by medial and lateral incisions, the joint capsule was opened, and the collateral ligament was identified. The periosteum was removed only at the area of drilling. A 2.5-mm guide wire was placed in the center of the epicondyle, perpendicular to the surface. A cannulated drill bit with a diameter of 11 mm was used to create a 12-mm deep drill hole. To avoid thermal damage to the bone, a drill speed of two rotations per second was used. After removing bone debris from the bone cavity the implants were inserted with a specially designed hollow cylinder impaction tool with an inner diameter of 7 mm and an outer diameter of 11 mm. This tool could slide over the implant while impacting the allograft into the 2.5 mm peri-implant gap. Finally, the top screw was mounted, and the soft tissues were closed in layers. The order in which the implants were inserted was (for all animals): (1) allograft; (2) allograft + low dose tobramycin; and (3) allograft + high dose tobramycin. In order to ensure uniform implant placement and minimal impaction technique variance, all surgeries were performed by the same experienced surgeon (JBA).

Postoperatively, the animals were given 1 g of Ceftriaxone intramuscularly for 3 days. A fentanyl transdermal patch (75 µg/h), lasting 3 days, was given as postoperative analgesic treatment. After surgery, the animals were allowed unlimited activity. All 12 animals were fully weight bearing within 3 days after surgery. Blood samples were collected day 1 and 3 postoperatively in order to quantify any systemic release of tobramycin. Serum levels of tobramycin were < 0.1 mcg/mL (detection limit) in all samples except one sample on day 3, measuring 0.3 mcg/mL. One animal died 5 days prior to completion of the 4-week observation period. An autopsy was performed; the cause of death was intestinal invagination. The veterinarian concluded that this was a natural cause of death and not due to the implants. The animal had been in normal health until that time, and as the observation

period was almost completed the animal was not excluded, and the bones were harvested and included in the analysis. The remaining 11 animals completed the observation period without signs of infection or other complications.

After the observation period of 4 weeks, the animals were sedated with Acepromazine, 0.5 mg/kg, anaesthetized using Propofol, 4 mg/kg, and euthanized with an overdose of hypersaturated barbiturate. The distal part of the femurs were dissected and stored at -80°C immediately after retrieval. Prior to dividing the bone into transverse sections for mechanical and histomorphometric analysis, the distal femora were thawed and the outermost 1 mm of the implant-bone specimen, containing the 11-mm top-screw, was cut off and discarded. The remaining 9 mm 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 (Exakt cutting/grinding system, Exakt Advanced Technologies, Norderstedt, Germany). The first section was cut to a thickness of 3.5 mm, stored at -20°C , and used for mechanical push-out testing. The second section, with the remaining 5.5 mm of the implant, was prepared for histomorphometry by dehydration in graded ethanol (70–100%) and embedded in methyl methacrylate (Art. 800590; Merck & Co, Inc®, Darmstadt, Germany). Previous studies have used a section thickness of 3.5 mm and found it sufficient to mechanically characterize the interface, leaving the remaining larger portion for histomorphometry, thereby improving the histomorphometric sampling. Using the vertical sectioning technique,¹⁷ four 30- μm -thick histological sections were cut parallel to the implant axis on a Leiden microtome which is a water-cooled, inner hole diamond saw microtome (KDG-95- MeProTech, Heerhugowaard, Holland) and stained with 0.1% Toluidine Blue (pH = 7) (Fluka, SigmaAldrich®, St. Louis, MO) for 10 minutes, rinsed, and mounted on glass. This preparation provided blue staining of noncalcified tissue and light blue staining of calcified tissue. The different types of calcified tissues, such as woven bone and lamellar bone were categorized on the basis of their morphological characteristics.¹⁸ No specimens were lost during preparation.

The thawed specimens were tested to failure by an axial push-out test on an MTS Bionics Test Machine (MTS® 858 Mini Bionix® Biomaterials Testing System, Eden Prairie, MN; Software: MTS® Test Star 790.00, version 4.0C). Testing was performed blinded and in one session. The specimens were placed with the cortical side facing upwards on a metal support jig with the 6-mm diameter implant centered over a 7.4-mm opening, assuring a 0.7-mm distance between the implant and the support jig, as recommended.¹⁹ A cylindrical test probe with a 5-mm diameter was mounted, and a preload of 2 N (Load) defined the contact position at the start of the test. The implant was pushed from the surrounding bone in the direction of the implant axis at a velocity of 5 mm/minute. Load (N) and implant displacement (mm) were continuously recorded. To account for differences in the thicknesses of the push-out specimens, the load data were normalized by an approximation of the implant surface area (implant diameter $\times$ height $\times \pi$). The ultimate shear strength (MPa) was determined from the maximum load applied until failure of the bone-implant interface and calculated as ultimate shear strength = $\text{Load}/\pi DL$, where D was the average diameter, and L was the height of the implant. Apparent shear stiffness (MPa/mm) was obtained from the linear section of the load–displacement curve and calculated as apparent shear stiffness = $(\text{Load}/\pi DL)/T$, where T was the displacement. The total energy absorption was calculated as the area under the load–displacement curve until failure²⁰

(Figure 3). Determination of the reproducibility for a push-out test was not possible due to its destructive nature.

Blinded histomorphometry was performed using the stereological software (newCAST® version 3.4.1.0; Visiopharm A/S, Hoersholm, Denmark). Volume fractions of new bone, bone graft, fibrous tissue, and marrow space were quantified by the point counting technique (3×3 points per field of view).²¹ On the implant surface, the area fractions of the same tissues were quantified by the line intercept technique (10 lines per field of view).¹⁷ Four vertical sections, representative of each implant, were sampled (Figure 4). The mean surface intercept count for each implant was 781 (range, 457–1091) and the mean gap volume point count was 1283 (range, 752–1749). Histomorphometric reproducibility was estimated from double measurements performed by the same person (intraobserver variation). An identical equipment and setup was used. One specimen (a total of four sections) was re-counted from the control group and from each intervention group. Reproducibility can be expressed as the within-subject coefficient of variation (CV)

$$CV = \frac{s}{\bar{x}}; s = \sqrt{\frac{1}{2 \times k} \times \sum_1^k d^2}$$

Where, $\bar{x}$ is the mean value of the first and the second estimate, k the number of double estimates and d the difference between the first and the second estimate.

We aimed at keeping the CVs under 20%, and a CV under 10% was considered as a high reproducibility. In this present study the CV was 8% regarding new bone ingrowth and ongrowth.

We computed means and standard deviations for the mechanical test values. All mechanical test data followed a normal distribution as evaluated by q-norm plots of the residuals. Differences between treatment groups were determined using repeated measures ANOVA followed by paired t -test where applicable (this was not the case for any of the variables). The absolute differences between treatment groups were presented as means with 95% CIs. Data from the histomorphometrical assessment were normally distributed for area fractions of new bone and marrow space, and volume fraction of new bone, bone graft, and marrow space. The area fraction of bone graft and fibrous tissue; and the volume fraction of fibrous tissue were not normally distributed, mainly due to many values close to zero. Difference between the normally distributed data were determined using the repeated measures ANOVA followed by paired t -test where applicable (this was not the case for any of the variables) and difference between the nonparametrically data were determined using Friedman repeated measures analysis of variance by ranks followed by Wilcoxon sign rank test where applicable (this was not the case for any of the variables). We used the Stata 11.2 software (StataCorp®, College Station, TX).

RESULTS

We found no differences in mechanical fixation of the implants as defined by shear strength, shear stiffness, and energy absorption for either dose of tobramycin (Table I).

New bone and bone grafts filled the gap in the control group and both intervention groups. No signs of infection or foreign-body reaction were observed. We observed no differences in any areal fraction parameters of tissue ingrowth between the two interventions and the control group (Table II) and none in the volume fraction in the gap around the implant (Table III). Also, we found no differences in total bone density (bone graft + new bone), control versus low; 29% versus 28%, $p = 0.54$; control versus high; 29% versus 26%, $p = 0.13$; low versus high; 26% versus 28%, $p = 0.38$.

DISCUSSION

Antibiotic impregnation of bone graft is much discussed in the literature over the past three decades.^{8,10,11,13,15,22,23} Small clinical trials using antibiotic-impregnated bone grafts in septic revision arthroplasty surgeries have been conducted and good control of infection has been achieved.^{6,14,15} However, toxicity studies have implied that high local concentration of antibiotic may critically impair osteoblast reproducibility.^{12,13} The purpose of this study was to investigate whether implant fixation would decrease by impacting morselized allograft bone impregnated with different quantities of tobramycin around experimental porous-coated titanium implants.

Our experimental implant model was designed to imitate impaction grafting of an uncemented endoprosthesis in human metaphyseal bone.²⁴ It was simple and had a high degree of control of parameters,²⁵ but the model had limitations. First, the implants were inserted extra-articularly and therefore not influenced by direct load or joint fluid. However, this unloaded model is highly reproducible,²⁵ and yields less biological variance than inherent in a weight-bearing model.²⁰ Second, skeletally mature dogs were chosen as experimental animals because their bone quality and trabecular density reflect human bone better than that of most other animals.²⁶ Dog bone, however, remodels three times more quickly than human bone, and all test animals were young and healthy with good bone quality and not the compromised bone seen surrounding a loose implant in humans. Third, the observation time of 4 weeks was chosen since the aim was to study influence of tobramycin impregnation on early implant fixation. Clinical RSA studies have shown that early implant subsidence is associated with late implant loosening.^{27,28} It is therefore of interest to study any experimental approach at an early time point. Furthermore, a longer observation time may equalize any disadvantage of early impaired mechanical fixation making it less likely to be detected within the power of this study. Fourth, due to the presence of the titanium implant, the thickness of the histological slides were not possible to prepare under >30 micrometer. Therefore, the histomorphometrical evaluation was performed on a tissue-level only, carrying the risk of being unable to detect cytotoxic effects of tobramycin on a cellular level. Fifth, while we exercised caution to minimize risk of antibiotic leakage between the intervention groups and the control group, we cannot ensure it did not occur. Separate instruments were used for each implant insertion and the order in which the implants were inserted (1. allograft; 2. allograft + low dose tobramycin; and 3. allograft + high dose tobramycin) was the same for all animals. Furthermore, postoperative measurements of serum tobramycin were all below detection limit (<0.1 mcg/mL) (except one measurement on day 3 (0.3 mcg/mL)), which indicates that haematogenous distribution between treatment groups would have been very low. Sixth, local tobramycin release was not

measured and we therefore cannot conclude on the potential antimicrobial benefits of adding tobramycin to the bone graft. Furthermore measurements of remaining quantity of tobramycin in the bone graft at euthanasia were not conducted, as all bone graft was used in the mechanical and histomorphometric analysis. Seventh, the quantity of absorbed tobramycin in the bone graft was not measured we therefore cannot know the exact levels of tobramycin in the gap but assume it to be corresponding well to the known added quantities.

The biomechanical push-out test showed no difference, although there was a trend toward decreased mechanical properties in all parameters with both doses of tobramycin. Although inconclusive, we think that it is worthwhile to take note of this trend. This is because Tobramycin-augmented allograft does not serve the purpose of augmenting implant fixation directly, but is rather added to protect the allograft from microbial growth. While this may seem feasible, the antimicrobial effect of adding tobramycin to allograft around implants is only documented in a few case series without control groups.^{6,14,15} None of these studies have evaluated the effect on implant fixation; the only parameter has been infection recurrence. Despite this lack of clear evidence, it is increasingly common clinical practice to add tobramycin or other antibiotics to the allograft for topical antimicrobial protection in procedures as different as joint replacement revisions and instrumented spinal fusions. In infectious revisions, recurrence of infection is higher. In such cases, a possible impairment of the early mechanical implant fixation may be a tolerable trade-off to an efficient antimicrobial protection. However; in cases where antibiotics are added routinely to the allograft, it is interesting to gain knowledge about potential adverse effects the antibiotics could have on implant fixation. No work has been published on this previously. The present study does not have sufficient power to prove similarity between the groups, and it can therefore not be used to document safety of this practice (a detection limit of 10% would require >85 animals and a limit of 5% would require >340 animals). The observed trend toward an impaired implant fixation with the use of tobramycin should be taken into consideration, at least in cases without manifest infectious loosening, where the benefit of adding topical antibiotics is unsubstantiated.

Histologically, the treatment groups were comparable with similar distributions of bone graft and new bone in the gaps. The histomorphometrical analysis revealed no differences between groups. This corresponds well to grafted defects studies performed in guinea pigs and canines where no difference in osteogenic activity between defects grafted with allograft bone with or without topical antibiotics was observed.^{8,23}

The present study is unable to provide evidence on whether the use of topical tobramycin with allograft is safe or whether it indeed can impair implant fixation. The tendency toward an impaired implant fixation warrants further preclinical studies. Its current clinical use should be weighed against its presumed positive effects on preventing infection in complicated revisions.

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FIGURE 1.

(A) The location of the gap implants in the distal femur is shown in a post-euthanasia radiograph. (B) The experimental porouscoated, titanium implant with the dimensions is shown. The last end-screw is mounted after impaction grafting of the gap. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 2.

(A) The implant is inserted and the peri-implant gap of 2.5 mm is empty. (B) The gap is impacted with morselized allograft bone with or without tobramycin. (C) The gap is closed with a top screw.

FIGURE 3.

On the left, a specimen is placed on a metal platform with central opening. The specimen thickness is 3.5 mm, the implant diameter is 6 mm, and the support hole is 7.4 mm (preload 2N, displacement velocity is 5 mm/minute). On the right, the load (N) displacement (mm) curve enables the calculation of ultimate shear strength (MPa), apparent shear stiffness (MPa/mm), and total energy absorption (KJ/m²).

FIGURE 4.

Histology: Representative histologic sections of the control group (A: Allograft) and intervention groups (B: Allograft + low dose tobramycin; C: Allograft + high dose tobramycin) from the same animal. Control: A-1: Overview of implant and bone graft; A-2: Thin fibrous tissue membrane in contact with implant; A-3: Allograft bone chip with ongrowth of new bone. Low dose: B-1: Overview of implant and bone graft; B-2: Allograft bone chip with ongrowth of new bone in contact with implant; B-3: Fibrous tissue membrane in contact with implant. High dose: C-1: Overview of implant and bone graft;

C-2: Thin fibrous tissue membrane in contact with implant; C-3: Allograft bone chip with ongrowth of new bone. Ipl = Implant, Nb = New bone, Ab = Allograft bone, Ms = Marrow space, Fib = Fibrous tissue. (Stain, toluidine blue; magnification, $\times 28$ (left), $\times 230$ (right). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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TABLE I.

Biomechanical Results

Groups (dose of Tobramycin, mg/mL)	Ultimate shear strength (MPa)	Apparent shear stiffness (MPa=mm)	Total energy absorption (KJ/m ²)
Control (0)	4.4 (2.5)	20 (12)	0.9 (0.6)
Low dose (200)	4.0 (1.8)	19 (8)	0.7(0.4)
High dose (800)	3.6 (1.6)	17 (8)	0.7 (0.3)
Absolute difference			
Low dose - control	-0.4 (-1.4; 0.5)	-1.0 (-6.8; 4.8)	-0.2 (-0.4; 0.0)
High dose - control	-0.8 (-2.8; 1.1)	-3.0 (-13; 6.9)	-0.3 (-0.7; 0.1)
High dose - low dose	-0.4 (-1.8; 1.1)	-1.9 (-8.4; 4.5)	-0.1 (-0.4; 0.3)
<i>p</i> value (ANOVA)	0.51	0.69	0.17

Biomechanical data are presented as mean values (SD), and the absolute group difference as mean with 95% CI

TABLE II.

Histomorphometrical Ongrowth: Tissue Area Fractions on Implant Surface

Surface fraction	New bone mean % (SD)	Fibrous tissue median % (IQR)	Bone graft, median % (IQR)	Marrow space mean % (SD)
Control	16 (4)	1 (0-2)	0 (0-0)	81 (6)
Low-dose	17 (6)	1 (0-7)	0 (0-0)	79 (6)
High-dose	17 (5)	2 (0-8)	0 (0-0)	79 (6)
Absolut difference				
Low dose - Control	-0.7 (-5.4; 4.0)			-2.2 (-6.1; 1.6)
High dose - Control	0.2 (-5.1; 5.4)			-2.4 (-7.2; 2.5)
High dose - Low dose	0.9 (-3.8; 5.6)			-0.1 (-3.1; 2.9)
<i>p</i> -value (ANOVA)	<i>p</i> = 0.92	<i>p</i> = 0.57	<i>p</i> = 0.47	<i>p</i> = 0.36
<i>p</i> -value (Friedman)				

IQR, Interquartile range.

TABLE III.

Histomorphometrical Ingrowth: Tissue Area Fractions on Implant Surface

Volume fraction	New bone Mean % (SD)	Fibrous tissue median % (IQR)	Bone graft, mean % (SD)	Marrow space mean % (SD)
Control	21 (4)	0 (0-0)	8 (3)	71 (6)
Low-dose	20 (6)	0 (0-0)	8 (3)	71 (8)
High-dose	19 (6)	0 (0-1)	7 (4)	74 (8)
Absolut difference				
Low dose - Control	-0.6 (-3.4; 2.4)		-0.4 (-2.0; 1.3)	0.4 (-2.6; 3.4)
High dose - Control	-2.1 (-5.4; 1.2)		-0.9 (-3.2; 1.4)	2.6 (-1.3; 6.6)
High dose - Low dose	-1.5 (-5.4; 2.5)		-0.6 (-2.5; 1.4)	2.3 (-2.1; 6.6)
<i>p</i> -value (ANOVA)	<i>P</i> = 0.40		<i>p</i> = 0.60	<i>p</i> = 0.28
<i>p</i> -value (Friedman)		<i>p</i> = 0.57		

IQR, Interquartile range.