Histological Evaluation of Bone Formation Adjacent to Dental Implants with a Novel Apical Chamber Design: Preliminary Data in the Rabbit Model

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ABSTRACT

Background: Wound healing events after implant placement will vary according to the extent of the necrotic zone.

Purpose: The goal of the present study was to evaluate bone healing around titanium implants with a novel apical chamber design.

Materials and Methods: Titanium implants grade 4 were turned with different apex design. Control implants had a self tapping design with centric cutting grooves. Test implants exhibited eccentric cutting grooves interconnected by a hollow chamber. A total of 60 implants were installed in the femur/tibia of 10 rabbits for histological analysis.

Results: After 1 week, immature bone formation started at the cortical level of the test implants associated to scalloped contours indicative of bone resorption. Control implants failed to show new bone formation, and the space within the threads was filled mainly by red blood cells and surgical debris. Bone contact values showed no difference after 1 week, and significant higher values for test implants showed likewise after 4 weeks compared with control implants in the tibia.

Conclusion: This experimental study verifies the beneficial effect of bone formation in the chamber at the apical part of the fixture coupled to a faster bone healing to implants placed in dense bone.

KEY WORDS: bone formation, bone-implant interface, implant design, osseointegration

INTRODUCTION

The success of dental implant rehabilitation is dictated by the integrity of bone-implant interface. Wound healing events that take place after implant installation follow a series of biological reactions related to numerous factors, and the surgical technique plays an important role. Minimally traumatizing surgical technique was originally reported to induce soft and hard tissue regeneration around implants placed in dogs. Higher pressure applied during drilling in combination with reduced irrigation was considered a traumatic technique, leading to mobility of the fixture, which was surrounded by a collagen-rich connective tissue capsule associated to soft tissue hyperplasia.[1] Traumatic surgery techniques will increase the temperature that may induce permanent bone tissue injury. A temperature higher than the threshold level of 47°C applied for 5 minutes and 50°C applied for 1 minute will result in significant change in the healing process that may ultimately not occur depending on the temperature and exposure time.[2,3] Inadequate drill design or too

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high drilling speed and pressure applied may result in values close or above the 47°C limit based on in vitro experiments.[4–6] In addition, more heat is generated when sites with extended depths are prepared and the highest temperature is observed in bone with higher density (cortical layer).[7,8]

Few reports indicate the relevance of the extent of necrotic bone related to the increased temperature during the drilling step. In a study performed in rabbits, a necrotic zone of 200 and 500 μm was observed by histological and histochemical observations, respectively.[9] A necrotic zone of 50 to 100 μm was found in the outer margin of the drilled hole after 3 weeks in the rabbit tibia where surgery followed the strict minimally traumatizing guidelines.[10] In the same study by Lundskog,[10] increasing temperatures of 60°C, 75°C, and 80°C resulted in a necrotic zone of 0.1, 0.9, and 1.1 mm, respectively, as measured by the absence of diaphorase enzyme activity.

The necrotic zone can extent up to 1 mm despite a careful surgical protocol, and the new bone formation will start from the periosteal and endosteal surfaces, not directly affected by the site preparation.[11] The endosteal and periosteal callus formation will reach the implant surface early as 2 weeks. At the interface, removal and replacement (remodeling) of the nonvital bone will occur between 2 and 6 weeks.[11]

Implant macrogeometry modifications are an alternative to modulate bone response. In a dog study, implants with microthreads on the crestal module exhibited higher bone contact compared with implants without microthreads, likely associated to a better stress distribution.[12] The influence of the thread design was evaluated in a rabbit model. After 12 weeks of healing, implants with square threads demonstrated greater bone contact and reverse torque values compared with standard V-shape and reverse buttress threads.[13] The overall implant design may contribute to better clinical results. Slightly tapered implants placed in type 4 bone resulted in higher initial stability associated to higher survival rates after 1 year follow-up.[14,15] The removal of the nonvital bone present at the bone-implant interface may represent an alternative to improve bone formation by reducing or minimizing the period of resorption leading to earlier bone deposition. The goals are (1) to reduce the amount of debris left by the drilling procedure and (2) to minimize the necrotic zone by removing the external walls at the cut bone surfaces. In the present study, a new apical chamber was tested in the rabbit tibia and femur after 1 and 4 weeks of healing. The aim was to compare the bone tissue healing around a (1) conventional self-tapping implant and (2) self-tapping implant with an apical chamber, with emphasis at the bone-implant interface and inside the apical chamber.

MATERIALS & METHODS

Implants

A total of 60 threaded implants were turned from c.p. Grade 4 titanium rods with an external diameter of 3.75 mm and a total length of 7.5 mm. Control and test implants were identical on the coronal and middle third of the implant. The only difference between the groups was the design on the apical third of the implants. Control implants had a self tapping apical design with centric cutting grooves aligned to the implant body. Test implants exhibited eccentric cutting grooves interconnected by a hollow chamber (Figure 1).

Animals and Surgical Technique

A total of 10 New Zealand White rabbits were used in the experiment. This study was approved by the local Ethical Committee of Huddinge University Hospital, Karolinska Institute, Sweden. The animals were adult (9 months of age) and weighted between 4 and 5 kg. The rabbits received one implant in each distal femoral metaphysis and two in each proximal tibial metaphysis (six implants/animal). Implant placement was randomized. The animals were kept in separate cages during the whole experiment. They had free access to tap water and standard diet. At surgery, general anesthesia was induced by intramuscular injections of fentanyl 0.3 mg/ml and fluanisone 10 mg/ml (Hypnorm Vet, Janssen Pharmaceutica, Beerse, Belgium) at an initial dose of 0.5 ml per kg body weight and intraperitoneal injections of diazepam (Stesolid, Dumex, Copenhagen, Denmark) at a dose of 2.5 mg per animal. Additional doses of Hypnorm at a dose of 0.1 ml per kg body weight were given every 30 minutes during the surgical procedure. The hind legs were shaved and cleaned with clorhexidin. Local anesthetic lidocaine hydrochloride (Xylocaine, AstraZeneca AB, Sodertalje, Sweden) at a dose of 1 ml was injected into each insertion site. The skin and fascial

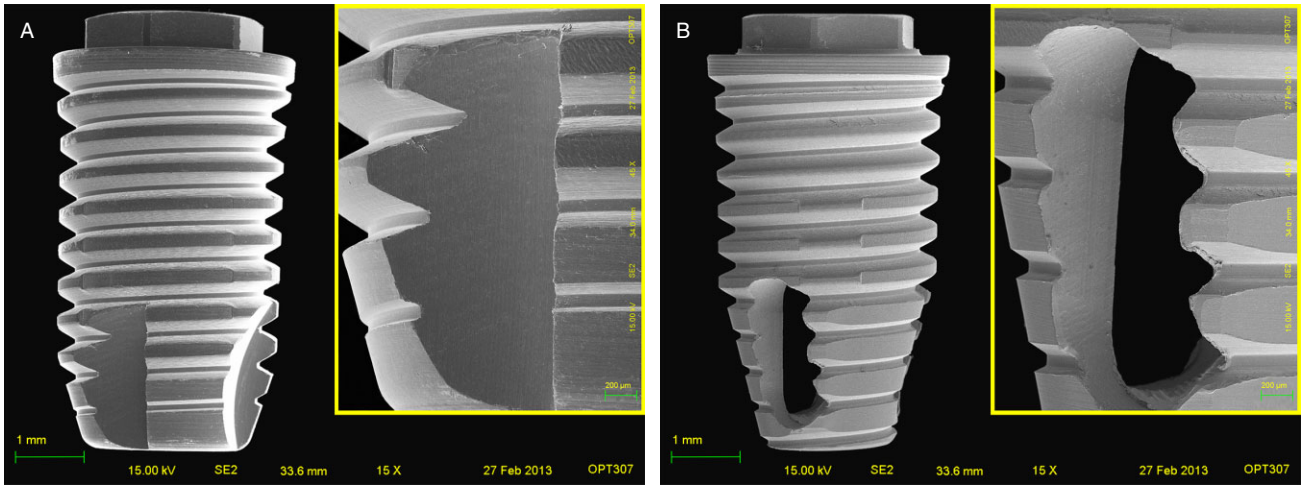

Figure 1 Scanning electron microscopy (SEM) micrograph of the control (A) and test (B) implants. The difference between the groups is limited to the apical third. Test implants reveal an apical hollow chamber with eccentric cutting edges. Coronal and middle thirds of control and test implants are identical.

layers were opened and closed separately. The fascial layers were sutured with resorbable sutures. The implantation holes were prepared by a round 2-mm drill followed by a 2.2-, 2.8-, and 3.3-mm twist drills at low rotary speed (800 rpm) with profuse saline cooling. The animals were allowed to bear their full body weight immediately after surgery.

Bone Response

The animals were sacrificed after 1 and 4 weeks with Pentobarbital Vet (Apoteket AB, Stockholm, Sweden) after sedation with 1.0 ml Hypnorm Vet. The implants and their surrounding tissues were removed en bloc and immersed in 4% neutral buffered formaldehyde. The specimens were dehydrated in graded series of ethanol and embedded in light curing resin (Technovit 7200 VLC, Heraeus Kulzer, Wehrheim, Germany). Undecalcified sections were cut and ground (Exakt Apparatebau GmbH, Norderstedt, Germany) to a thickness of about 20 µm and stained with toluidine blue and 1% pyrogin-G. Examinations were performed with a Nikon 80i microscope (Nikon Instruments, Melville, NY, USA) equipped with an image software analysis (NIS-Elements BR 3.2, Nikon, USA) using 1X to 100X objectives for descriptive evaluation and morphometrical measurements. The qualitative analysis aimed at describing the early bone formation events at the control and test implants. The histomorphometrical evaluations comprised measurements of the degree of bone implant contact and bone area limited by the first and third coronal thread at a distance of approximately 600 µm from the thread valleys.

Statistical Analysis

The multiple regression mixed models were used to study the effect of group on the outcomes, adjusting for endpoint and site. The calculated effect sizes were adjusted for multiple comparisons using Dunnett–Hsu's correction. All analyses were performed using SAS, release 9.2 (SAS Institute Inc., Cary, NC, USA). p Values $<.05$ were considered significant.

RESULTS

Histological Evaluation

The implant site in the femur consisted mainly of trabecular bone, whereas tibial sites were characterized by a cortical layer of 1.5 mm in height. The original bone trabeculae in the femur were in contact with the top five threads, and the cortical layer in the tibia was in contact to the 2 to 3 top threads. The apical part of test and control implants in the tibia (approximately 2.5 mm) was inside the bone marrow cavity. The apical chamber from the test implants was cut in different orientations during the histological sectioning, and both the cutting grooves and the gap between the cutting grooves (corresponding to the entrance of the chamber) could be observed.

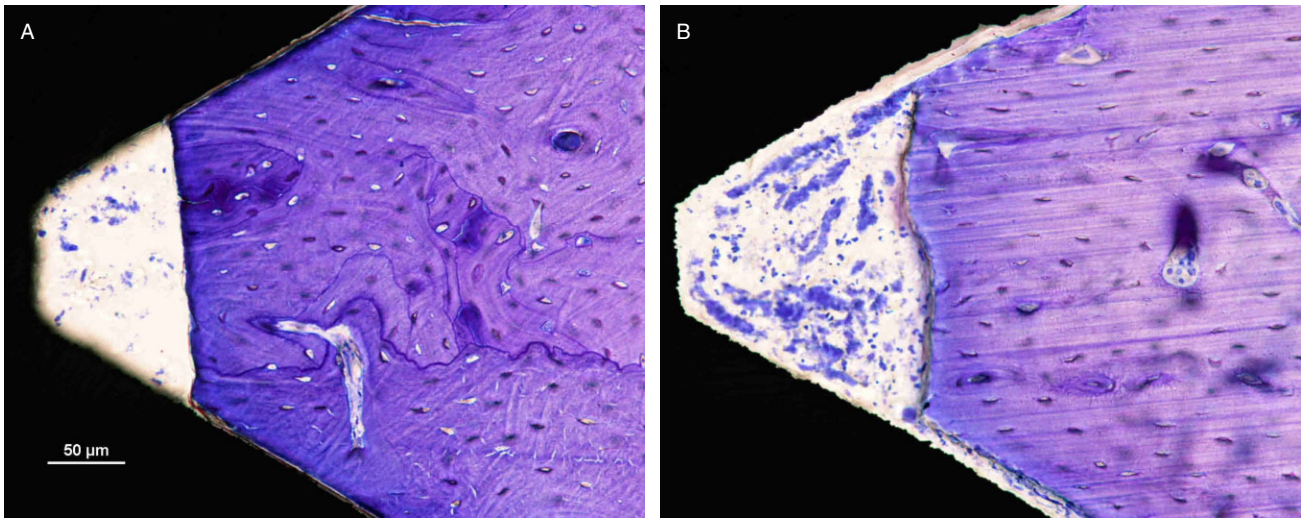

Figure 2 Control (A) and test (B) implants after 1 week of healing (20×). Bone cut surface is intact on the control implant with no signs of bone resorption or deposition. Space between the thread and the bone surface is filled by few red blood and inflammatory cells (A). Bone surface adjacent to test implants shows signs indicative of bone resorption and early mineralization already started (B).

1 Week

Light microscopy of 1 week specimens demonstrated signs indicative of early bone resorption on the cut bone surface of test implants. Osteoclasts could not be detected, but the shallow scalloped contour suggests active bone resorption. Immature woven bone formation started within the thread region of the test implants at the cortical level, apparently not connected to the bone or implant. At the control implants, bone surface did not reveal clear signs of bone resorption, and the space within the threads was filled by clot with red blood cells undergoing disintegration and surgical debris (Figures 2 and 3). Both implants showed typical endosteum reaction leading to new bone downgrowth from the third to fourth thread, and no difference on the tissue development stage could be detected between the groups at this region (Figure 3). After 1 week of healing, intense new bone formation was observed inside the test implant chamber. The new bone formation was observed on the perimeter of the preexisting bone

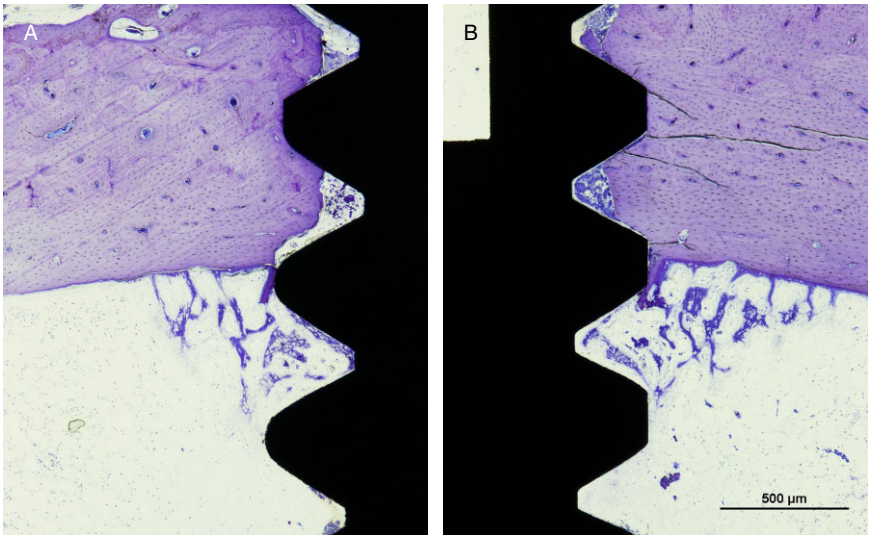

Figure 3 Control (A) and test (B) implants after 1 week of healing. Similar endosteum bone downgrowth was observed in both implants. Red blood cells and surgical debris are found on the control implants at the cortical level (A). Drilling edge can be observed associated to immature bone formation within the threads of the test implants (B).

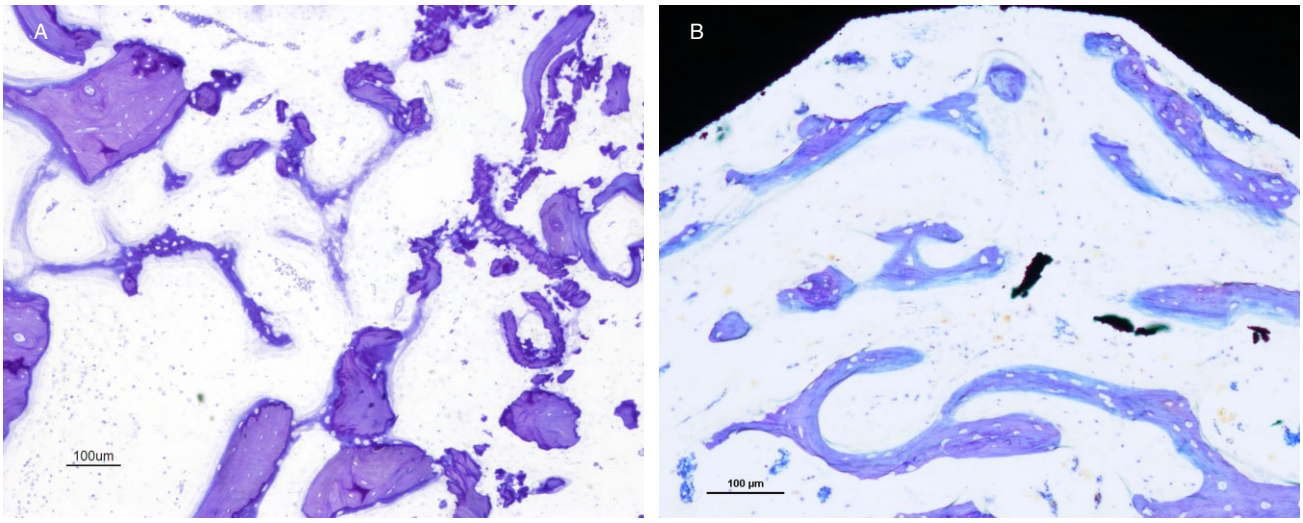

Figure 4 Bone shaves inside the chamber at (A) 1- and (B) 4-week intervals. Signs of bone resorption associated to new bone formation were found around the bone shaves after 1 week. After 4 weeks, bone shaves were remodelled, and newly formed bone was present inside the chamber.

shaves, interconnecting the different pieces through osteoid seams surrounded by osteoblasts (Figure 4).

4 Weeks

At 4 weeks, the newly formed mineralized tissue contains osteocytes and osteobleast seams indicating continuous mineralization of the tissue. At this stage, bone healing was characterized by the appearance of vascular units inside the threads. The newly formed bone was apparently more mature at the test implants with centric osteocytes positioned in the lamellae around the canal. Less organized tissue was found at the control implants, where bone was at the final stages of mineralization and there was no evident sign of lamellar bone (Figure 5). Inside the chamber of the test group, bone shaves were remodeled, and new bone formation was present in similar amount compared with 1 week (Figure 4). Only few larger original bone shaves could be found inside the chamber. In some sections, the new bone formation inside the chamber was found to be connected to the

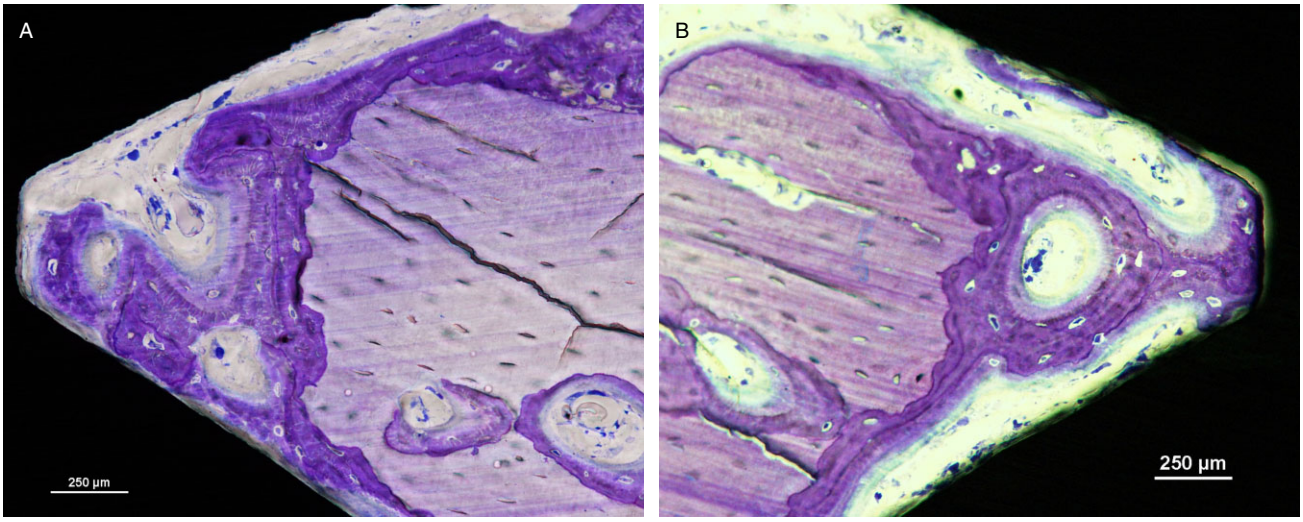

Figure 5 Higher bone formation was observed inside the threads of test implants (A) compared with control in the tibia (B). The new bone formation along the interface occurred from an area with signs of resorption. (A) Bone tissue around control implants reveals structures compatible to early lamellar structures where osteocytes are not centric organized. (B) Bone formation was apparently more mature on the test implants, where lamellar structures surrounded by centric osteocytes can be observed.

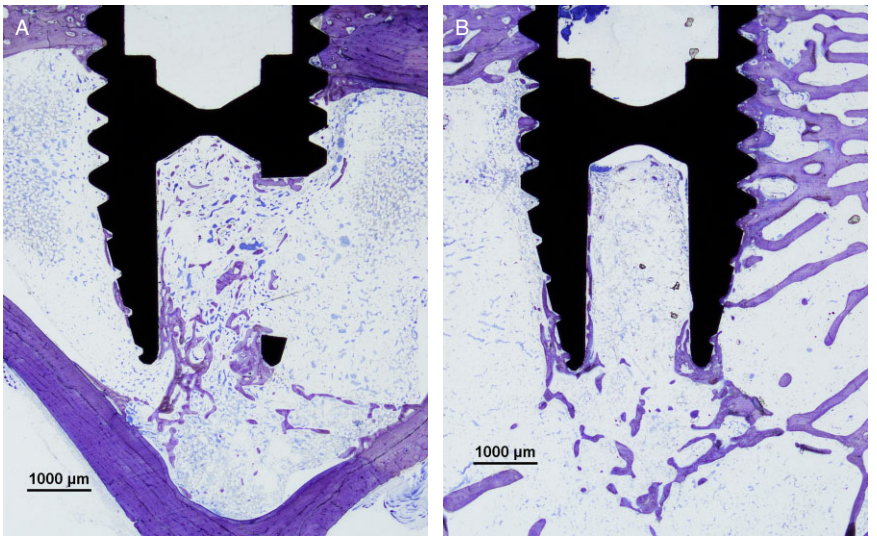

Figure 6 New bone formation inside the chamber was connected to the lower cortical of the tibia (A) and to the trabecula on the femur (B). Bone shaves were remodeled and only few larger structures could be found after 4 weeks.

original trabecula in the femur and to the lower cortical in the tibia (Figure 6).

Histomorphometrical Analysis

Similar bone–implant contact was observed after 1 week of healing both in the tibia ($p = .97$) and femur ($p = .45$) (Table 1). After 4 weeks, higher bone–implant contact values to test implants were detected in the tibia ($p = .04$) and similar values in the femur ($p = .84$). Bone area values were similar between control and test implants at the different intervals (Figure 7).

DISCUSSION

The findings from the present study indicate a novel approach to improve bone healing around titanium implants. The presence of an apical hollow chamber with eccentric cutting grooves apparently minimized the effect of trauma from surgery, resulting in improved wound healing as observed by the bone development stage and bone–implant contact values in dense bone. After 1 week of healing, initial solitary woven bone formation (early mineralization) was observed inside the threads of test implants at the cortical level. Control implants failed to show any signs of early mineralization, and the threads were mainly filled by coagulum at the same interval. After 4 weeks, the presence of osteons surrounded by lamellar structures with centric osteocytes indicates a faster organization of bone tissue at the implants with the hollow chamber. Control implants showed similar bone area, with less organized tissue and reduced bone–implant contact values. The observations

TABLE 1 Results from Bone Contact Measurements

Week	Site	Group	Mean (95% CI)	Dunnett–Hsu *p* Value
1	Femur	Control	17.6 (3.9–31.2)	0.97
		Test	17.7 (9.7–25.8)	
	Tibia	Control	11.7 (9.0–14.4)	0.45
		Test	13.0 (10.3–15.7)	
4	Femur	Control	35.4 (18.1–52.0)	0.84
		Test	36.6 (29.9–43.4)	
	Tibia	Control	22.5 (18.2–26.8)	0.04*
		Test	28.0 (24.3–31.8)	

*Denotes significant difference. Mean values (95% CI).
CI, confidence interval.

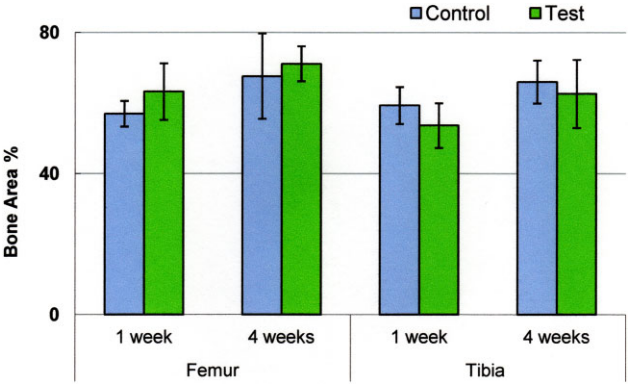

Figure 7 Similar bone area values were calculated for both implant groups after 1 and 4 weeks of healing.

of the current experiment were in agreement with the results reported by Sennerby and colleagues (1993).[16] The authors reported that no signs of bone resorption or formation were observed at the cortical passage after 1 week of healing, similar to the present findings at the control implants. Early bone formation after 1 week of healing was mainly observed at the endosteum area, again in agreement with the present results observed at the control implants. However, in the present study, early mineralization was observed at the test implants at the cut bone surface already after 1 week, not reported by Sennerby and colleagues.

The surgical protocol was identical for both test and control implants. In addition, the implant design is identical on the first, second, and third, and the only obvious difference is the presence of the hollow chamber and the eccentric cutting edges in the apex. Furthermore, the surface properties of both groups were identical as the implants were turned from titanium rods of identical specification. Thus, the only variable that could explain the enhanced bone formation at the test implants is the different macrogeometry of the apex. The presence of the chamber could affect bone contact as a result of the different apical design, and no effect could be related to the wound healing process. This hypothesis is not supported by the histomorphometrical results after 1 week of healing, where similar bone contact and bone area values were observed. Such results clearly indicate that the improved bone formation is explained by biological events that take place at the interface of the chamber implant. Similar bone formation starting from the endosteum was observed between the two implant groups at the 1- and 4-week interval. Bone downgrowth started early after 1 week and continued until 4 weeks with a similar appearance and volume. The lack of differences found in this region is explained by the identical (1) surgical protocol and (2) surface properties of the implants. Bone downgrowth from the endosteum is caused by the disruption of blood vessels[11,17] and can also be affected by the surface properties of the implants.[18,19]

The current findings revealed early mineralization on the cortical passage of the rabbit tibia already after 1 week of implant installation. At this time point, new bone formation is expected in the threads bellow the cortical layer (inside the bone marrow, as a result of the endosteum injury during drilling) or adjacent to noncompact bone. The slower wound healing activity observed adjacent to compact bones may be related to the extension of the nonvital zone (indicative of tissue trauma). This nonvital zone formed after the surgical procedure has been reported by different authors and described as an area with empty osteocyte lacunae or osteocytes exhibiting altered morphology. Histological observations of the wound healing events on trabecular bone of rats (maxilla) showed a 100-μm zone of affected osteocytes.[20–22] However, when the implants were placed in cortical bone of rats, the zone of altered osteocytes extended up to 400 μm.[23] Bone remodeling activity seems to vary according to the width of the affected region. Trabecular bone resorption started at 3 days, whereas bone formation started after 5 days.[20–22] Cortical bone resorption was observed only in some specimens after 7 days, and bone formation was observed after 14 days,[23] indicating a delayed remodeling activity in the cortical bone compared with trabecular bone. The findings reported by Ohtsu and colleagues (1997) in cortical bone of rats[23] were similar to the results obtained in rabbit cortical bone.[24] A region of 200 to 400 μm of altered osteocytes could be detected and bone resorption started after 7 days and bone deposition after 14 days.[24] Despite the many differences between the two models used,[23,24] the presence of a similar extent of altered osteocytes was related to similar remodeling events on the cortical bone of rats and rabbits. The extent of the nonvital zone was not evaluated in the present nondecalcified sections and may be a possible explanation for the enhanced bone formation to the test implants. Future studies should investigate the extent of the nonvital zone associated to implants with hollow chambers on decalcified sections.

The bone shaves collected during implant placement were rapidly remodeled, and only few large shaves were detected after 4 weeks. In some sections, the new bone formation taking place inside the hollow chamber was interconnected to the surrounding preexisting bone. Such findings are of great clinical interest if such results could be reproduced in patients. The bone shaves trapped inside the chamber may trigger new bone formation to the implant apex, resulting in increased bone contact values. The clinical relevance of the present findings remains to be explored.

In conclusion, this experimental study verifies the beneficial effect of bone formation in the chamber at the apical part of the fixture coupled to higher bone contact values at the bone-implant interface in dense bone.

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