Bone Regeneration with Rabbit Bone Marrow–Derived Mesenchymal Stem Cells and Bone Graft Materials

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Purpose: *This study compared the bone regeneration response of different bone graft materials inside canals within anodized titanium implants in cortical and cancellous bone.* ***Materials and Methods:*** *Upper and lower transverse canals were created in anodic oxidized–surface titanium implants to serve as sites for cortical and cancellous bone regeneration, respectively. The canals were filled with bone graft materials—rabbit bone marrow-derived mesenchymal stem cells and platelet-rich plasma, xenograft, or alloplast (micro-macroporous biphasic calcium phosphate)—or left empty (as a control). Eighty implants were surgically placed into the tibiae of 20 New Zealand white rabbits. After 4 and 12 weeks of healing, histomorphometric analysis was performed to measure the newly formed bone areas (NBs) inside the canals.* ***Results:*** *Inside the upper canals, the bone graft groups provided significantly higher NBs than the control (no graft). However, there was no significant difference in NBs between the bone graft groups. Inside the lower canals, no significant difference in NBs was shown among the all groups. The NBs inside the upper canals were significantly greater than those inside the lower canals in all groups after 4 and 12 weeks, respectively.* ***Conclusions:*** *In the cortical bone, there was significant difference in bone regeneration between the control and the bone graft groups. However, there was no significant difference among the bone graft groups in cortical and cancellous bone regeneration. There was significant difference in bone regeneration between the cortical and cancellous bone regions in the all groups using the titanium canal model.* Int J Oral Maxillofac Implants 2012;27:1389–1399

Key words: *bone graft material, bone regeneration, mesenchymal stem cell, tissue engineering, implant canal model*

Bone replacement graft materials have played an important role in regenerative dentistry for many years. Among the various techniques to reconstruct deficient alveolar bone, autogenous bone grafting (autograft) has become a predictable, well-documented surgical approach and the gold standard of care.[1] However, the use of autografts is associated with substantial morbidity, including infection, malformation, pain, and loss of function.[2] In addition, autografts are limited in supply, are occasionally not suitable for the proposed reconstruction because of poor tissue quality, and can be extremely difficult to shape.[3,4] These disadvantages have led to a continuous search for suitable bone substitutes.

An ideal bone substitute should be biologically compatible, nonsupportive of local pathogens or cross-infection, and osteogenic. In addition, it should match the physical composition of natural bone trabeculae and provide scaffolding for new bone ingrowth.[5] That is, materials that are osteogenic (the cells within a donor graft synthesize new bone at the implantation site), osteoinductive (new bone is formed through the active recruitment of host mesenchymal stem cells [MSCs] from the surrounding tissue, which differentiate into bone-forming osteoblasts), osteoconductive (vascularization and new bone formation in the transplant), and highly biocompatible are necessary.[6]

Various bone graft materials, such as allografts, xenografts, and alloplasts (substitutes), are being extensively studied as alternatives to the harvesting of autogenous bone.[7] Allografts are tissues taken from

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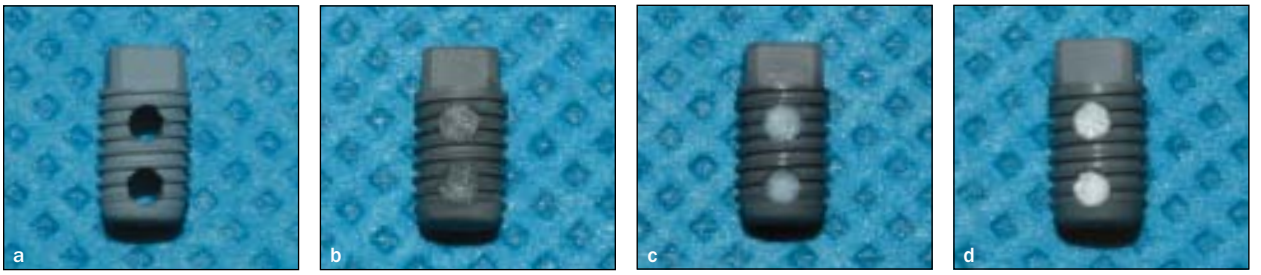

Fig 1 An image of a threaded commercial pure titanium implant with two transverse canals. The implants had a 5-mm threaded body (pitch height, 0.6 mm), a 2-mm unthreaded square top, and a 3.75-mm outer diameter. The implants had two transverse canals, each 1.5 mm in diameter, that were separated by 1.0 mm. The upper and lower transverse canals were filled with various materials (*left to right*): nothing (control), MSCs/PRP, Bio-Oss, and MBCP.

individuals of the same species as the host and are used as a source of type 1 collagen. However, such grafts have the disadvantages of limited supply and potential infectivity (eg, AIDS, hepatitis).[8] In comparison, xenografts are derived from other species. Bovine hydroxyapatite (HA) is a type of xenograft material obtained from calf bones from which all organic components have been removed. The remaining inorganic structure is an excellent source of calcium and phosphate. It also provides a natural architectural matrix. The bone-conductive properties of bovine HA have been demonstrated in previous studies.[9,10] Alloplastic materials, such as synthetically manufactured porous HA and tricalcium phosphate, are available in unlimited supply but have the disadvantage of possessing osteoconductivity without osteoinductive properties.[11,12]

A promising alternative approach to bone regeneration was established by the identification of certain multipotential cells among the stromal cells of bone marrow.[13] Many studies of tissue engineering have been conducted, and many successful studies using MSCs have been reported.[14–16] An MSC is an undifferentiated cell that can self-replicate and differentiate into various tissues such as bone, cartilage, nerve, and tendon. It also has many abilities and functions, including immune depression. Studies have confirmed that an undifferentiated MSC can proliferate and differentiate into a desired tissue in a specific environment.[17,18] Studies on the regeneration of bone defects using MSCs have also been reported.[19,20]

Bone regeneration by means of tissue engineering can solve problems and disadvantages encountered in autogenous bone grafts, such as limited bone quantity and the need for additional operations. The main factors of bone regeneration using tissue engineering are MSCs, growth factors, and the presence of a three-dimensional scaffold.[21,22] Yamada et al[3,23] used MSCs as isolated cells and platelet-rich plasma (PRP) as the growth factor and scaffold ("tissue-engineered injectable bone").

In the present study, bone regeneration inside canals that had been created within anodized implants were filled with different grafting materials (MSCs/PRP, xenograft, alloplast) and evaluated. The purposes of this study were: *(1)* to compare the values of newly formed bone areas (NBs) among the different bone graft materials, and *(2)* to compare the NBs between the cortical and cancellous bone areas in all groups.

MATERIALS AND METHODS

Implant Preparation (Anodic Oxidation) and Animals

Eighty screw-shaped implants of commercially pure titanium (grade 4) were prepared. The implants had a total length of 7 mm (5 mm threaded and 2 mm unthreaded at the coronal portion), an outer diameter of 3.75 mm, and a thread pitch height of 0.6 mm. The head of the implant was square. Two transverse canals, each 1.5 mm in diameter and 1.0 mm apart, passed through the threaded part of the implant. The implants were treated with anodic oxidation at 300 V in an aqueous electrolytic solution of 0.02 mol/L calcium glycerophosphate and 0.15 mol/L calcium acetate. The implant was attached to the anode, and stainless steel was used as the cathode. All procedures were executed at room temperature, with a total time for anodic oxidation of 3 minutes per implant. The implants were then rinsed ultrasonically with distilled water and absolute alcohol for 5 minutes and dried. The anodized surface morphology was determined using field emission scanning electron microscopy (S-4700, Hitachi) at a 15-kV accelerating voltage. The anodized implants were sterilized in ethylene oxide gas before use. Four different groups were prepared according to the following procedures:

- Group 1: The two transverse canals were left empty as a control (Fig 1a).
- Group 2: The two transverse canals were filled with rabbit bone marrow–derived MSCs that had been mixed with PRP (tissue-engineered injectable bone) (Fig 1b).

- Group 3: The two transverse canals were filled with xenografts (inorganic bovine bone matrix, Bio-Oss, Geistlich Biomaterials) (Fig 1c).
- Group 4: The two transverse canals were filled with alloplasts (micro-macroporous biphasic calcium phosphate [MBCP]) (Fig 1d).

Twenty New Zealand white rabbits aged 6 to 9 months and weighing 3 to 3.5 kg each were used in this study. All of the animals were treated and handled in accordance with the "Recommendations for Handling of Laboratory Animals for Biomedical Research" compiled by the Committee on the Safety and Ethical Handling Regulations for Laboratory Animal Experiments in the College of Dentistry at Seoul National University (approval number: SNU-060615-1).

Bone Replacement Graft Materials

Tissue-Engineered Injectable Bone. Figure 2 provides a diagram of the MSCs/PRP creation process. The rabbits were housed in separate cages and fed a standard diet. Four weeks prior to implant placement surgery, bone marrow was harvested via iliac crest aspiration. During surgery, general anesthesia was induced via an intramuscular injection of 10 mg/kg Zoletil (Vibac Laboratories) and 0.15 mg/kg Rompun (Bayer Korea). The skin and cortical bone of the iliac crest were punctured with a bone marrow aspirator (Klima-Rosegger modified Luer Lock, Unimed) under local anesthesia with lidocaine (Yuhan) containing 1:100,000 epinephrine. After it was confirmed that the bone marrow aspirator was fixed, 8 to 10 mL of bone marrow was aspirated with a 10-mL syringe that contained 1 mL of heparin (Choongwae Pharm) according to a previously reported method.[24]

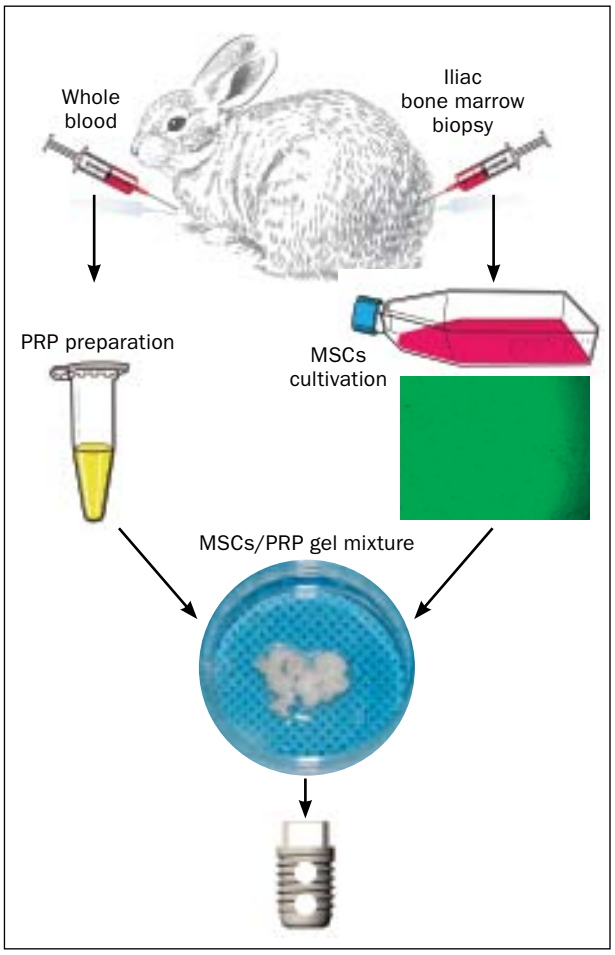

Fig 2 Schematic diagram of the MSCs/PRP group.

The mixture of aspirated bone marrow and 15 mL of basic culture medium (low-glucose Dulbecco modified Eagle medium, 10% fetal bovine serum, and a penicillin-streptomycin mixture) were collected in a T175 flask (Nalge Nunc). The medium was changed after 24 hours to remove nonadherent cells. Subsequently, the medium was changed three times a week. Rabbit bone marrow–derived MSCs were cultured with a technique used in previous studies.[3] Primary culture was performed in a humidified atmosphere of 95% air with 5% carbon dioxide at 37°C for 10 to 12 days. After primary culture, the rabbit bone marrow–derived MSCs were released from their culture substratum using 0.05% trypsin–ethylenediaminetetraacetic acid (Sigma-Aldrich). The cells were concentrated via centrifugation at 1,500 rpm for 5 minutes at room temperature and counted. The cells were divided into T175 flasks and subcultured. A quantity of 1×10^7 cells was then placed into each T175 flask, and the cells were cultured in osteogenic induction medium[25] (0.1 μmol/L of dexamethasone, 0.05 mmol/L of L-ascorbic acid 2-phosphate, and 10 mmol/L of β-glycerophosphate; Sigma-Aldrich) for 2 weeks. Following this, the differentiated MSCs were confirmed through the detection of alkaline phosphatase (ALP) activity using *p*-nitrophenyl phosphatase as a substrate. ALP activity was assayed as the release of p-nitrophenol from *p*-nitrophenyl phosphate at a pH of 10.4. The reaction was stopped after 30 minutes using 2 N sodium hydroxide, and the absorbance measurement was performed at 405 nm using an enzyme-linked immunosorbent assay reader (PowerWave X 340, BioTek Instruments). The cells were trypsinized, and 1×10^7 cells/mL were prepared for bone regeneration.

The PRP and gel were prepared according to the method developed by Yamada et al,[3] in which 30 mL of whole blood were aspirated with a 50-mL syringe that contained 3 mL of 3.2% sodium citrate under general anesthesia. The sample was then centrifuged at 1,100 rpm for 10 minutes (UNION32R, Hanil Science Industrial). The supernatant that contained the buffy

coat was centrifuged again at 2,500 rpm for 5 minutes to produce a 3-mL pellet of PRP. The PRP was then stored in a conventional shaker (Unimax 1010 DT, Heidolph Instruments) until use.

After powdered thrombin (bovine thrombin, 10,000 U, Sigma-Aldrich) was dissolved into 10 mL of 10% calcium chloride solution, 100 μL of the thrombin/calcium chloride mixture was transferred to a 1-mL syringe. Separately, 600 μL of PRP and rabbit bone marrow–derived MSCs (1×10^7 cells/mL) were collected with a 1-mL syringe. The two syringes were connected with a three-way stopcock (Hyupsung Medical), and the contents were mixed. Within 30 to 60 seconds, the contents assumed a gel-like consistency, because the thrombin affected the polymerization of fibrin to produce an insoluble gel. The MSCs/PRP admixture was injected into the transverse canals of the implants using a syringe.

Other Bone Graft Materials (Xenograft and Alloplast). Anorganic bovine hydroxyapatite (Bio-Oss) is the inorganic component of bovine bone (ie, the minerals) and has a 75% to 80% porosity. All of the organic material from these bones is removed using a stepwise annealing process (up to 300°C), followed by a chemical treatment (sodium hydroxide). The remaining material is porous hydroxyapatite bone chips, the average particle size of which is 0.25 to 2 mm. This material is similar to human cancellous bone and has a crystal size of approximately 10 μm in the form of cortical granules.[26]

Synthetic biphasic calcium phosphate (MBCP, Biomatlante) is a ceramic composed of two phases of calcium phosphate: 60% HA ($Ca_{10}[PO_4]_6[O]_2$) and 40% β-tricalcium phosphate (β-TCP; $Ca_3[PO_4]_2$). The particle sizes of the granules range between 0.5 and 1.00 mm. There are two types of porosity in MBCP: microporosity (30% to 33%, with pores ranging from 1 to 10 μm) and macroporosity (50%, with pore diameters from 300 to 600 μm). The total porosity ratio is about 70%, with micropore sizes similar to those observed in trabecular bone.[27]

Surgical Implant Placement

Prior to surgery, the operating sites were shaved and carefully washed with iodine solution. Local anesthesia (1.0 mL of 2% lidocaine including 1:100,000 epinephrine, Yu-han) was injected into the tibiae aseptic conditions.

Using sterile surgical techniques, an incision was made in the skin to expose the proximal aspect of each tibia, and the muscles were dissected to allow elevation of the periosteum. The flat surface on the lateral aspect of the proximal tibia was selected for implant placement. The holes were drilled with a low-speed rotary instrument under constant irrigation with sterile saline.

A total of four implants, one from each group, were randomly placed in the right and left tibiae of each rabbit and penetrated only the first cortical layer. The upper canals of the implants were located in the cortical bone region, and the lower canals were located in the marrow. These canals served as sites for the evaluation of bone ingrowth.[28] The surgical site was closed in layers: the muscle and fascia were sutured with resorbable suture material (chromic catgut, Ethicon) and the skin was sutured with black silk (Mersilk, Ethicon).

After surgery, all of the rabbits received 50 mg/kg Kanamycin (Dong-A) via intramuscular injection. The rabbits were sacrificed via intravenous injection of potassium chloride (Daihan Pharm) at the scheduled time.

Specimen Preparation and Histomorphometric Analysis

Ten rabbits each were sacrificed after 4 and 12 weeks for histomorphometric analysis. All 80 implants and the surrounding bone were removed en bloc; fixed in neutral buffered formalin; dehydrated in 70%, 90%, 95%, and 100% alcohol; and embedded in light-curing resin (Technovit 7200 VLC, Kulzer). The embedded implants were divided longitudinally with a saw (Exakt, Exakt Apparatebau). The sections were ground to a thickness of approximately 30 μm, as described by Donath and Breuner,[29] and were stained with 1% toluidine blue to highlight the collagen content.

The histomorphometric analysis was performed with the aid of an Olympus BX51 microscope (Olympus) connected to a computer onto which the program Kappa Imagebase (Kappa Opto-Electronics) was loaded. All of the measurements were calculated under 100× magnification. The percentages of newly formed bone area (NB), graft materials area (GM), and marrow spaces (MS, ie, the soft marrow spaces containing fatty marrow tissue, connective tissue spaces, and empty spaces). The variables were calculated as follows (Fig 3):

NB inside the canals (%) = (newly formed bone area in the canal)/(total area of the canal) × 100

GM inside the canals (%) = (graft particles area in the canal)/(total area of the canal) × 100

MS inside the canals (%) = 100 – (NB inside the canals + GM inside the canals)

Statistics

Statistical analyses were carried out using SPSS 12.0 (IBM). Tests of normality and equality of variances were applied, and no violations of those basic assumptions were observed. A mixed-model analysis of variance was used to compare the amounts of newly formed bone inside the canals of the four groups and to

control for the random effects of the individual rabbits. Post hoc analysis was done with the Tukey test. All values were considered significant when $P < .05$.

RESULTS

Histologic Findings

After 4 Weeks. Survey images of the implants confirmed monocortical insertion into the central part of the tibia. The upper canals of the implants were positioned in the first cortical region of the bone in all sections, and the lower canals were positioned in the marrow regions. Both the upper and lower canals demonstrated bone ingrowth, with more bone in the upper canals in all groups (Fig 4).

Figure 5 shows detailed images of the 4-week specimens. The upper canals in group 1 demonstrated newly formed islands of bone and ingrowth into the canal from the outside. In general, the newly formed bone was located on the lateral side of the canal, often in broad close contact with the implant surface of the upper canal (Figs 5a and 5i). Fatty marrow spaces were observed mostly in the lower canals. Only a small amount of newly formed bone could be detected in group 1 (Figs 5e and 5m).

In the upper canals in group 2, the trabeculae of newly formed bone were scattered in the canals. The newly formed bone appeared to be woven or of irregular architecture. The remnants of the scaffold and fibrous connective tissues were dispersed in the canals (Figs 5b and 5j). In the lower canals, sparse and thin trabeculae of bone were rarely observed. An isolated island of newly formed bone was observed in the central part of the canal. The fibrous patchlike scaffold was transversely spread out in the canals (Figs 5f and 5n).

In the upper canals in group 3, Bio-Oss particles could be seen embedded in the newly formed bone. The Bio-Oss particles served as a conductor for the newly formed bone, which interconnected the individual particles. The sizes and shapes of the Bio-Oss particles were used to indicate the differences from the newly formed bone. While the newly formed bone appeared long and thin, the particles of bovine HA were short, thick, and polygonal in shape (Figs 5c and 5k). In the lower canals, a smaller amount of newly formed bone was observed, mostly with a pattern similar to that seen in the upper canals (Figs 5g and 5o).

In the upper canals in group 4, the MBCP particles were surrounded by newly formed bone. No gaps were present at the bone-particle interface, and the bone was in contact with the particles. The MBCP particles presented marked staining differences versus the newly formed bone. The MBCP granules were sharp-edged and relatively dark in color, whereas the newly formed bone particles were long, with relatively obscure boundaries (Figs 5d and 5l). In the lower canals, a small amount of newly formed bone was observed on the particle surface (Figs 5h and 5p).

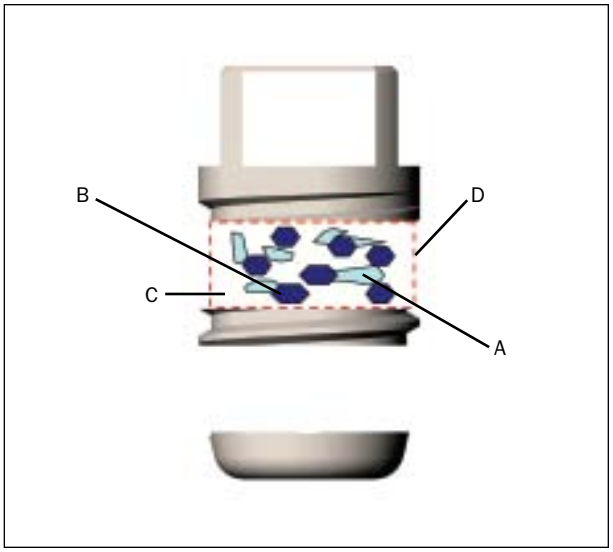

Fig 3 Schematic diagram of the histomorphometric analysis. A = Newly formed bone areas in the canal; B = graft material areas in the canal; C = marrow spaces; D = total area of the canal. Percentage of NB inside the canals (%) = A/D × 100 (%); percentage of GM inside the canals (%) = B/D × 100 (%); percentage of MS inside the canals (%) = C/D × 100 (%) = 100 – [(NB) + (GM)].

After 12 Weeks. The histological views at low magnification (Fig 6) showed that the upper canals were situated in the first cortical region of the bone and the lower canals in the marrow region in all groups. Figures 7 and 8 show the detailed specimens after 12 weeks of healing.

In the upper canals in group 1, newly formed islands of bone showed a configuration extending toward the central part of the canal from the lateral side. Portions of the trabeculae of newly formed bone were in close contact with the superior and inferior anodized surfaces of the upper canal (Figs 7a, 7i, and 8a). Most of the fatty marrow spaces were observed in the lower canals. A thin layer of newly formed bone was observed along the implant surface of the canal (Figs 7e and 7m).

In the upper canals in group 2, islands of newly formed bone were located in the canals. The newly formed bone formed trabeculae, which were dispersed in the scaffold. The remnants of scaffold and fibrous connective tissues were also dispersed in the canal (Figs 7b, 7j, and 8b). In the lower canals, strands of fibrous scaffold were observed running parallel to the canal. Islands of thin trabeculae of newly formed bone were sparsely observed within the fibrous patchlike scaffold (Figs 7f and 7n).

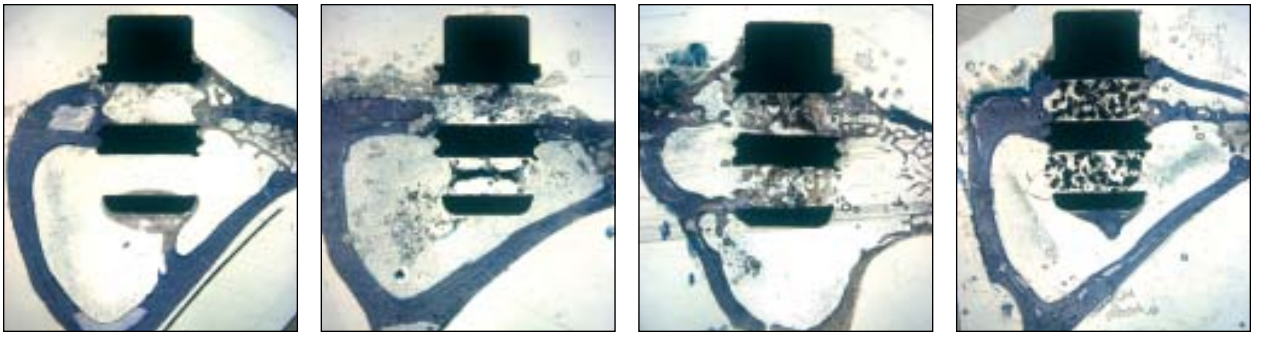

Fig 4 Histologic views of the threaded titanium implants with two transverse canals after implantation in the rabbit tibia for 4 weeks (toluidine blue; magnification ×12.5). (*Left to right*) Control, MSCs/PRP, Bio-Oss, MBCP.

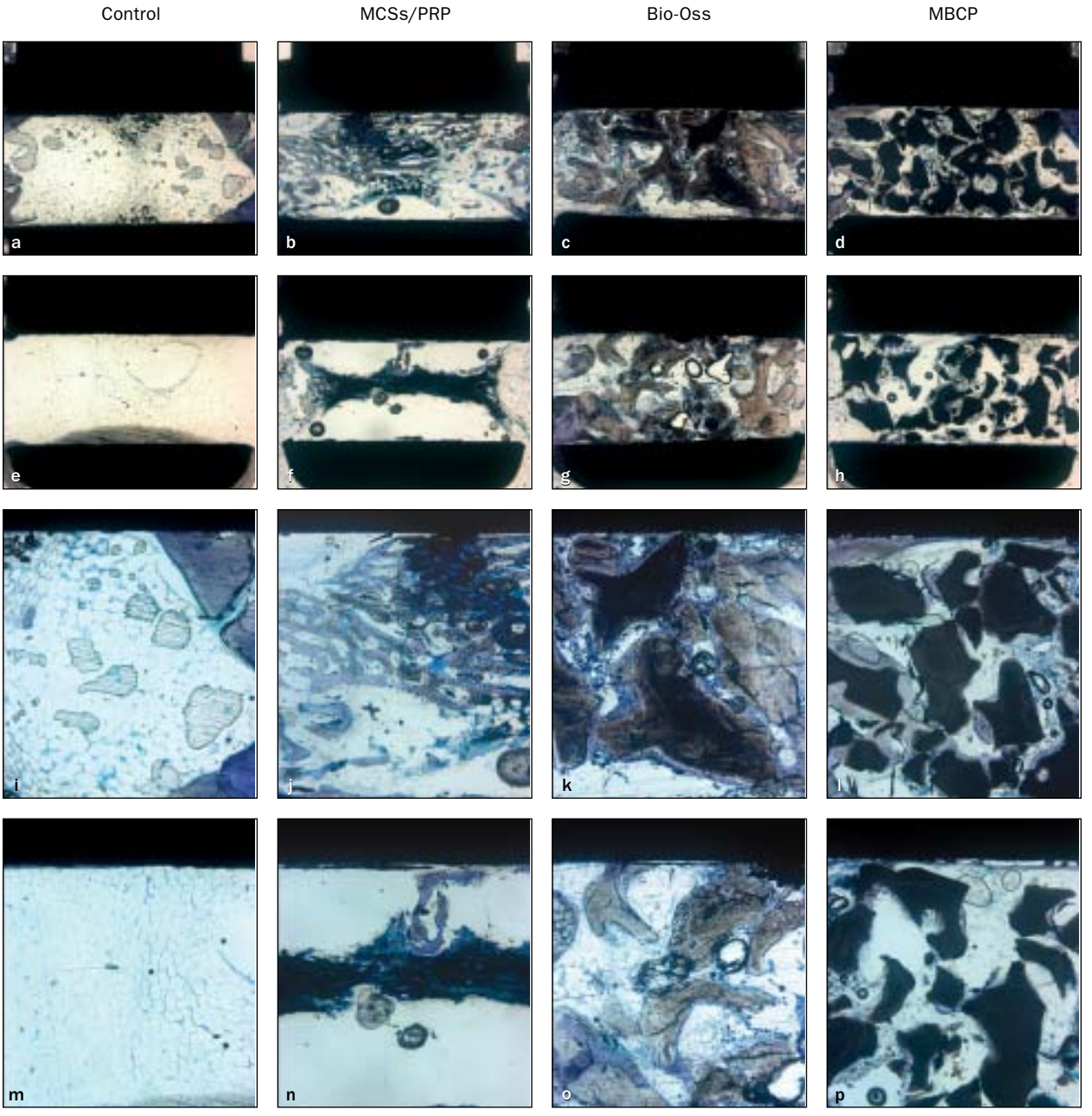

Fig 5 Histologic views after 4 weeks. *Columns, left to right*: Control, MSCs/PRP, Bio-Oss, MBCP; *rows, top to bottom*: upper canals, ×40; lower canals, ×40; upper canals, ×100; lower canals, ×100 (toluidine blue).

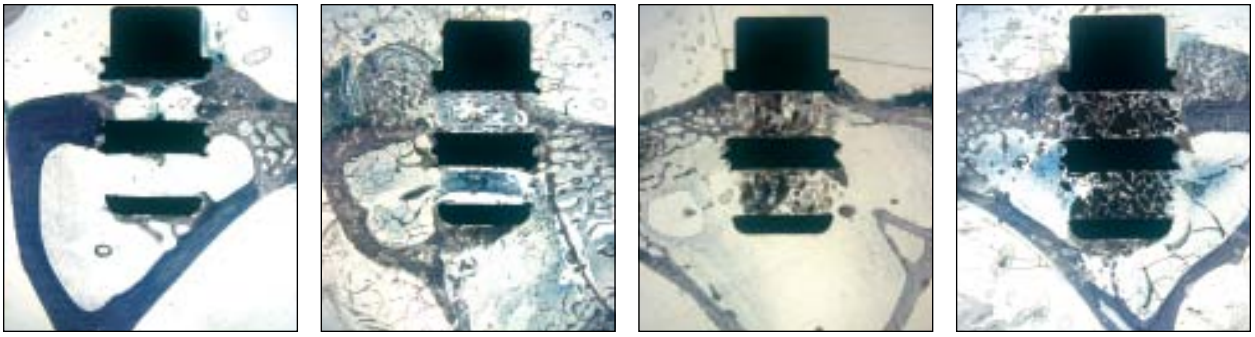

Fig 6 Histologic views of the threaded titanium implants with two transverse canals after implantation in the rabbit tibia for 12 weeks (toluidine blue; magnification ×12.5). (*Left to right*) Control, MSCs/PRP, Bio-Oss, and MBCP.

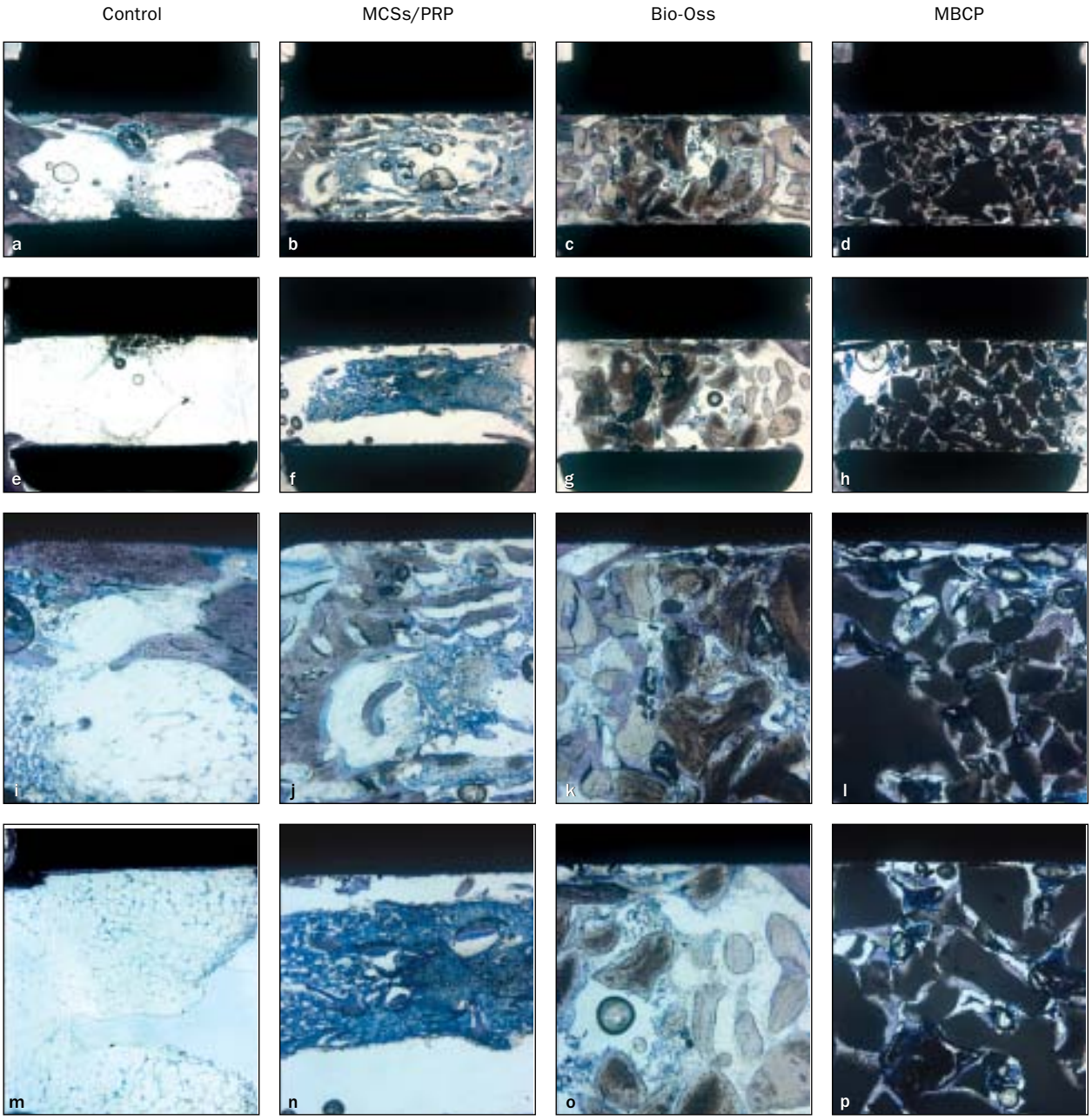

Fig 7 Histologic views after 12 weeks. *Columns, left to right*: Control, MSCs/PRP, Bio-Oss, MBCP; *rows, top to bottom*: upper canals, ×40; lower canals, ×40; upper canals, ×100; lower canals, ×100 (toluidine blue).

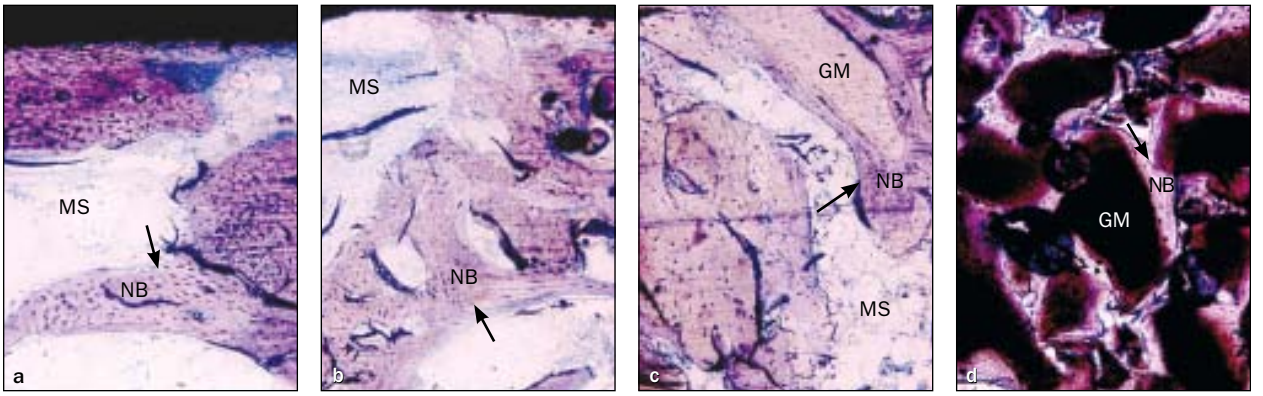

Fig 8 High-power histologic views inside the upper canal after 12 weeks in the (*a*) control, (*b*) MSCs/PRP, (*c*) Bio-Oss, and (*d*) MBCP specimens (toluidine blue; magnification ×200). Newly formed bone (NB and *arrows*), graft material (GM), and marrow space (MS) were observed. The trabeculae of NB can be seen in the control and MSCs/PRP sites. The particles of GM were surrounded by NB in the Bio-Oss and MBCP specimens.

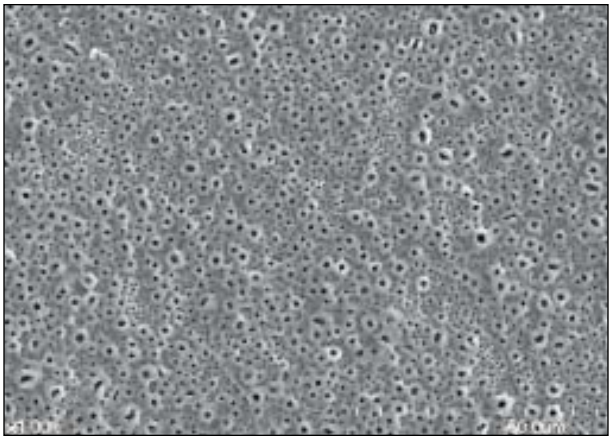

Fig 9 Scanning electron micrograph of anodized implant surface. The surface had a layer of interconnecting pores composed of small craters with holes at the centers (magnification ×1,000).

In the upper canals in group 3, Bio-Oss particles were incorporated in various proportions within newly formed bone. The surfaces of the particles were in contact with newly formed bone, which interconnected the trabeculae (Figs 7c, 7k, and 8c). In the lower canals, a small amount of newly formed bone was observed around the Bio-Oss particles (Figs 7g and 7o).

In the upper canals in group 4, MBCP particles were surrounded by bands of newly formed bone. No gaps were present at the bone-particle interface, and the bone was in contact with the particles (Figs 7d, 7l, and 8d). In the lower canals, the graft materials were in contact with thin bands of newly formed bone. This apposition of newly formed bone in the lower canals exhibited a similar pattern to that observed in the upper canals but was present in smaller quantities (Figs 7h and 7p).

Generally, for the upper canals in groups 3 and 4, bone grew inward from the preexisting cortical bone into the grafted area within the canal.

Table 1 Histomorphometric Values Inside the Canals at 4 Weeks

Location/ group	NB (%)	GM (%)	MS (%)
Upper canal			
Control	10.28 ± 2.08	–	89.72 ± 2.08
MSCs/PRP	14.96 ± 2.38	–	85.04 ± 2.38
Bio-Oss	17.83 ± 4.62	60.53 ± 5.29	21.64 ± 7.37
MBCP	17.11 ± 5.29	59.32 ± 4.52	23.58 ± 7.06
Lower canal			
Control	3.45 ± 1.61	–	96.55 ± 1.61
MSCs/PRP	3.92 ± 1.83	–	96.08 ± 1.83
Bio-Oss	4.78 ± 1.49	59.19 ± 5.43	36.03 ± 6.43
MBCP	5.03 ± 1.76	58.01 ± 5.06	36.96 ± 5.01

Values are given as means ± standard deviations.

Table 2 Histomorphometric Values Inside the Canals at 12 Weeks

Location/ group	NB (%)	GM (%)	MS (%)
Upper canal			
Control	13.63 ± 3.90	–	86.37 ± 3.90
MSCs/PRP	18.94 ± 3.22	–	81.06 ± 3.22
Bio-Oss	22.11 ± 4.18	56.02 ± 5.63	21.87 ± 5.65
MBCP	23.03 ± 5.11	57.84 ± 4.68	19.13 ± 7.61
Lower canal			
Control	5.07 ± 1.31	–	94.93 ± 1.31
MSCs/PRP	5.76 ± 1.44	–	94.24 ± 1.44
Bio-Oss	6.33 ± 1.50	57.60 ± 4.70	36.07 ± 3.75
MBCP	6.95 ± 1.46	58.10 ± 4.50	34.95 ± 4.90

Values are given as means ± standard deviations.

Implant Surface Morphology and Histomorphometric Results

The surfaces of anodized titanium implants had the typical appearance of uniformly porous surfaces composed of small craters with holes at the centers (Fig 9). The NB, GM, and MS inside the upper and the lower canals of the implants are summarized in Tables 1 and 2.

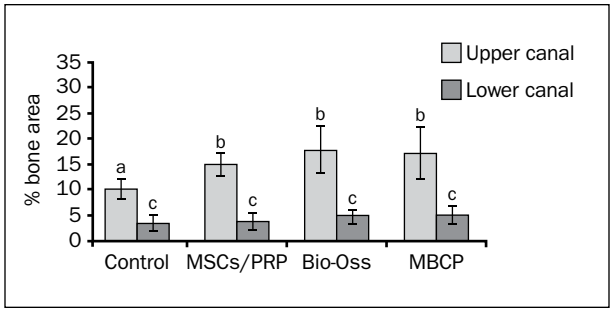

Fig 10 Areas of NB inside the upper and lower canals implanted into the rabbit tibiae for 4 weeks. Values are means ± standard deviations (n = 10).

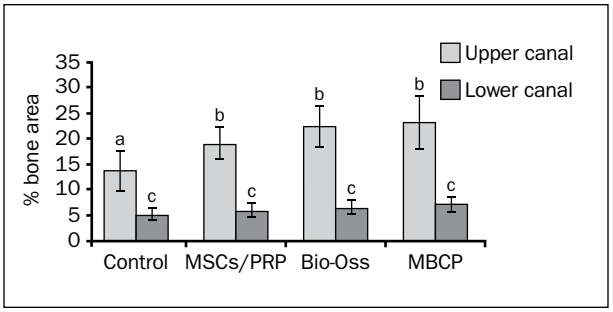

Fig 11 Areas of NB inside the upper and lower canals implanted into the rabbit tibiae for 12 weeks. Values are means ± standard deviations (n = 10).

After 4 weeks, groups 2, 3, and 4 showed significantly higher NB percentages than group 1 in the upper canals (group 2 > group 1, $P = .047$; group 3 > group 1, $P = .001$; group 4 > group 1, $P = .002$). However, there was no significant difference between groups 2, 3, and 4. There were no significant differences between all groups inside the lower canals ($P = .112$) (Fig 10).

After 12 weeks of healing, in the upper canals, groups 2, 3, and 4 showed significantly higher NB percentages than group 1 (group 2 > group 1, $P = .034$; group 3 > group 1, $P < .0001$; group 4 > group 1, $P < .0001$). There was no significant difference between groups 2, 3, and 4. There was no significant difference between the all groups inside the lower canals ($P = .064$) (Fig 11). In addition, the mean values for NB inside the upper canals were significantly higher than those of the lower canals in all groups after 4 and 12 weeks of healing ($P < .0001$).

DISCUSSION

In this study, the authors examined the bone regeneration with various bone replacement materials inside canals created within anodized implants. One of the graft materials used was tissue-engineered injectable bone (ie, MSCs/PRP). The MSCs/PRP group in this study had rabbit bone marrow–derived MSCs and a fibrin network scaffold of PRP gel as the injectable bonelike material. The PRP gel offers numerous advantages: (*1*) plasticity, (*2*) flexibility, (*3*) three-dimensional scaffolding, (*4*) reduced invasiveness because of the delivery through a syringe, (*5*) absence of toxicity, and (*6*) lack of an immune reaction.[3] In the osseous defect model, a mixture of MSCs and PRP produced well-formed mature bone and good neovascularization compared to the control (unfilled) defects.[3] According to Ito et al,[5] the MSCs/PRP possess osteogenic characteristics and may repair bone defects. In this study, the MSCs/PRP group also exhibited better bone regeneration inside the upper canals than did the control after 4 and 12 weeks according to histomorphometry when transverse canals were used to carry the bone graft materials.

Despite the osteogenic capacity of MSCs, they cannot be used as spatial fillers for bone defects.[30] MSCs are usually applied to a scaffold, such as PRP gel. Although the reason for active bone regeneration in the MSCs/PRP group remains unknown, it may be a consequence of the growth factors containing PRP. Therefore, it seems that bone regeneration inside the canals could be promoted by MSCs/PRP because of the presence of osteogenic cells and osteoinductive factors in the MSCs/PRP mixture.

Other bone replacement materials, such as Bio-Oss and MBCP, have been used increasingly often to simplify surgical procedures. Xenogeneic bone matrices and synthetic HA/β-TCP share several advantages: (*1*) no donor site is required, (*2*) unlimited supplies of the materials are available, and (*3*) the materials are easy to handle.[31] A majority of the currently available matrix materials for bone grafting are osteoconductive materials that support bone formation by acting as scaffolds for angiogenesis, cell recruitment, and ultimately osteogenesis by host cells. Variables that affect the osteoconductive nature of a bone graft include porosity, surface topography, and chemical composition.[32] Bio-Oss possesses important properties, such as biocompatibility and osteoconductivity, that make it a good scaffold for the ingrowth of host cells.[9,10] In the present study, transverse canals filled with Bio-Oss also displayed osteoconductive bone regeneration. Histologic observation revealed that Bio-Oss particles were incorporated and interconnected by trabeculae of newly formed bone, thereby demonstrating high osteoconductivity. This arises from an interconnecting pore system and from physical and chemical properties similar to those of human cancellous bone.[26]

A combination of the two primary forms of calcium phosphate has been studied to take advantage of the rapid resorption of β-TCP and the inert scaffold of dense HA. In a histologic study, biphasic calcium phosphate supported active bone replacement from surrounding bone, possibly as the result of a macrophage trigger.[33]

In the MBCP group in this study, the particles showed intimate contact with newly formed bone and provided a substrate for bone-forming cells. This material also showed osteoconductive properties. Other experimental studies have detected newly formed bone in the peripheral macropores of the MBCP particles.[11,12]

Ideally, bone substitutes should maintain their mechanical stability and volume during healing and then be completely resorbed and replaced by newly formed bone.[34] Strictly speaking, the MSCs/PRP admixture was the graft material. However, histologic observation showed that the PRP scaffold was biodegradable and was sparsely dispersed in a shrunken pattern, similar to fibrous connective tissue. Except for the newly formed bone, this fibrous scaffold was included in the marrow space area. Therefore, the GM inside the canals could not be measured in the MSCs/PRP group. On the other hand, in the Bio-Oss and MBCP groups, the particles were barely absorbed after 12 weeks of healing and remained as the substratum for the regeneration of bone inward to the canal from the preexisting cortical bone. It seems that the role of a space filler or space maintainer inside the canal was weak in the MSCs/PRP group because of the gel-like consistency, fibrous dispersion, and shrinkage pattern of the material.

Because the healing of severed bone tissue is a complicated process and apparently involves different steps in the cortical and spongious parts of a surgical site,[35] in the present study, the implants were surgically placed so that the transverse canals were parallel to the different regions of bone (cortical/cancellous bone). Generally, bone comprises outer cortical bone and inner cancellous bone, and the transverse canals could therefore serve as sites for evaluation of bone ingrowth.[28] In this study, the upper canals were positioned within the cortical region of the bone and the lower canals were located in the marrow region, so that the bone ingrowth of the upper canals represented cortical bone regeneration, while the bone ingrowth of the lower canals represented cancellous bone regeneration. In this study, the upper canals showed significantly higher NBs than the lower canals in all groups. However, there were no differences in NBs inside the lower canals located in the cancellous bone of the rabbit tibiae among all groups. These differences may be explained by the nature of the bone in the areas of the canals. Lu et al[36] reported that bone growth in rabbits was more pronounced in cortical bone than in the marrow sites. Generally, in rabbit tibiae undergoing implantation experiments, a larger amount of bone is observed in the threads in the old cortical region than in the threads in the marrow cavity.[37]

In addition to the ratio of cortical/cancellous bone of the rabbit tibia, the bone density and mechanical stability of the canals seem to be affected by the difference between the bone regions. The stiff and compact outer holding of the cortical bone provides a firm mechanical interlocking of the upper canal, which is important for bone regeneration. The relatively smaller existing bone fragments and weakness of the cancellous bone seem to affect the different bone responses.

In this study, the MSCs/PRP mixture, Bio-Oss, and MBCP inside the upper canals led to more newly formed bone compared to the control group sites. In spite of the fact that the osteogenic and osteoinductive properties of the MSCs/PRP are beneficial to bone regeneration,[3,5] there was no difference between the three bone graft materials in both upper and lower canals in this study. This may be a consequence of the canal model.

This implant canal model seems to be a useful tool for quantifying bone regeneration. The canals represent a well-defined space that is easy to prepare. The canal dimension is uniform, which allows quantitative measurement of the newly formed bone area inside the canal. These canals also can be filled with various bone grafting materials. The canals served as a space for bone ingrowth evaluation as well as a repository for the materials and carrier. When the canals are placed within the bone, bone regeneration is not affected by the environment of the overlying tissue and mechanical stimuli.[38,39]

However, the canals have potentially different biologic environments. Inside the canals, the oxygen tension, vascularity, cell activity, etc, are different versus a lesion in bone or beside the threads of an implant. According to Boyan and coworkers,[40] environmental factors such as oxygen tension help determine whether mesenchymal cells will differentiate into fibroblasts, chondrocytes, or osteoblasts. The environment inside the canals may be unfavorable to bone regeneration adjacent to the implant surface from a bone healing perspective. The canal or chamber itself is an osteoconductive structure,[41] so it also affected the bone ingrowth of all the tested sites. Thus, within the limitations of this canal model study, the results indicate that there was no difference between the bone graft materials. Further studies are required to clarify the osteogenic and osteoinductive capacities as well as the osteoconductive effects of the bone graft materials in the bone defects around implants in clinical bone regeneration.

CONCLUSIONS

In this study, anodized implants were designed with upper and lower transverse canals that were filled with different bone graft materials. The regeneration of cortical and cancellous bone with these bone substitutes inside anodized implant canals was evaluated using histomorphometric analysis of rabbit tibiae after 4 and 12 weeks. From this canal model study, the following conclusions can be drawn:

1. The newly formed bone percentages in the cortical bone were significantly greater in the grafted groups than in control sites, but there was no significant difference among the graft groups.
2. In cancellous bone, there was no significant difference in new bone percentage among all groups.
3. The new bone percentages in cortical bone were significantly higher than those in cancellous bone in all groups.

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