THE EFFECT OF AUTOLOGOUS PLATELET-RICH PLASMA (PRP) ON BONE FORMATION AROUND DENTAL IMPLANT IN THE RABBIT : A HISTOMORPHOMETRIC AND REMOVAL TORQUE STUDY

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Platelet-rich plasma(PRP) has been known to increase the rate and degree of bone formation by virtue of growth factors in concentrated platelets. Although its great healing effect on bone defect or pre-implantation site preparation in conjunction with bone substitute has been reported, the effect associated with implant is unknown. The purpose of this study was to investigate the effect of PRP on rapid osseointegration of endosseous dental implants in the rabbit tibiae. Twenty two adult female New Zealand white rabbits, weighing approximately 2.7-3.3kg, were used for this study. Twelve of the 22 animals were used for histomorphometric analysis and ten of the 22 were for removal torque test. Each animal received two implants in each tibia (two treated with PRP and two as control) and was given fluorochrome intramuscularly. For histomorphometric analysis, rabbits were divided into four groups according to the healing period. At 1 week, 2 weeks, 4 weeks and 8 weeks postoperatively, each three animals were sacrificed serially and the amount and rate of bone formation around dental implant were examined on the undecalcified sections under fluorescent microscope, polarized microscope and light microscope connected to a personal computer equipped with image analysis system. For removal torque test, rabbits were divided into two groups and removal torque tests were performed at 4 weeks, 10 weeks after implant placement. In total, 88 screw shaped, commercially pure titanium implants (Neoplant, Neobiotech, Seoul, Korea) were used in this study. Labeling pattern reflected differences of two groups in bone formation rate at each period. Histomorphometrically, PRP group showed significantly higher bone volume within threads compared to control group at 2 weeks (70.30$\pm$4.96% vs. 50.68$\pm$6.33%; $P < .01$) and 4 weeks (82.59 $\pm$5.94% vs. 72.94$\pm$4.57%; $P < .05$). PRP group at 1, 2 and 4 weeks revealed similar degree of bone volume formation comparable to control group at 2, 4 and 8 weeks, respectively. On the other hand, while PRP group showed higher bone-implant contact (47.37$\pm$8.09%) than control group (33.16$\pm$13.47%) at 2 weeks, there were no significant differences between PRP group and control group for any experimental period. Removal torque values also showed no significant differences between PRP group and control group at any experimental period ($P > .05$). These findings imply that PRP could induce rapid, more bone formation around implant during early healing period and get faster secondary stability for reducing healing period, though it has not induced bone maturation enough to resist functional loading.

Key Words
PRP, platelet-rich plasma, growth factor, implant, labeling, fluorochrome, histomorphometry, removal torque

Replacing the lost tooth immediately without edentulous period and getting fast osseointegration in every situation are current challenging aims of implant treatment modality.

Although a three- to six-month unloading period after implant placement is generally accepted protocol to ensure osseointegration[1], it seems not absolute prerequisite to osseointegration capable of supporting implants. Several factors are known to influence the anchorage of an implant in bone: biocompatibility of the material, design, surface quality, status of the bone, surgical technique and implant loading conditions.[2] Of which, primary implant stability at placement is a mechanical phenomenon that is related to the local bone quality and quantity, the type of implant and placement technique used. In contrast, secondary implant stability is the increase in stability attributable to bone formation and remodeling at the implant/tissue interface and in the surrounding bone.[3]

Therefore, the length of healing period is directly related to the bone formation rate, since the stability of implants is related to the properties of surrounding bone.

To shorten the healing period, many attempts were tried. Surface modification of implant is one of the effective methods to induce bone formation at the interface.[4-6] Another possible method to accelerate bone regeneration around dental implant is the use of bone growth factors. Growth factors are considered to act at an early stage of bone regeneration cascade by regulating the proliferation, differentiation, motility and matrix synthesis of nearly all cell types.[7] Indeed, recent studies demonstrated 5 fold to 10 fold increase of new bone formation in growth factor treated sites.[8]

Several authors reported the importance of proteins and growth factors found in the autologous fibrin adhesive(AFA) and platelet concentrates.[9-13] In the plasma, fibronectin, fibrin and other growth factor are included.[10] On the other hand, platelets produce and release some kinds of growth factors like PDGF, TGF-β. Thus these factors concentrated in platelet-rich plasma (PRP) are believed to have the potential to hasten healing and to improve bone quality. Tayapongsak *et al.*[9] used the AFA to cancellous bone graft as a career in the reconstructive mandibular surgery and reported 50% earlier remodeling process. In 1998 Marx *et al.*[11] showed 1.62 to 2.16 times higher radiographic maturation rate in the PRP group than in the control group by the application of PRP into autogenous bone grafts.

Their results suggested the possibility of early implant placement and/or early loading with enhanced bone quality and quantity after PRP treatment. Really, PRP has been applied to bone defect of human,[9-11,14-25] and majority of published reports stated PRP improved bone healing and contributed to early function. But, most of authors have focused on the use of PRP in conjunction with autologous bone or bone substitute[9-11,15-20,22-31] and for pre-implant site preparation.[15-17,19-25,29-30] Effects of platelet concentration on the capacity of bone formation without substitute and around implant have not been demonstrated clearly, yet. Therefore author tested the hypothesis that PRP alone, adsorbed to implant without bone substitute would enhance bone regeneration for earlier healing and loading.

The purpose of this study was to investigate the effect of PRP on rapid osseointegration of endosseous dental implants in the rabbit tibiae. It was investigated whether the PRP, adsorbed onto implant surface and placed into bone bed, enhanced the rate and degree of bone formation around implant during the healing period. Thus two experimental series were designed : First, to compare bone-implant contact as well as peri-implant bone volume at various time intervals, histomorphometric analysis and fluorescent mi-

croscopic observation of bone formation were performed. Second, to demonstrate the mechanical strength of new bone formed, removal torque of unscrewing was evaluated.

REVIEW OF LITERATURE

Growth factors

Polypeptide growth factors are natural cell products regulating the chemotaxis, proliferation, differentiation, motility and matrix synthesis of nearly all cell types.[7,32,33]

Those currently believed to contribute to the regeneration of bone, include platelet-derived growth factor (PDGF), transformig growth factor-β (TGF-β), Insulin-lke growth factor (IGF-I), fibroblast growth factor (FGF) and bone morphogenetic protein (BMP).[16] Growth factors have effects ranging from mitogenicity (IGF-I, PDGF-BB) to increasing activity of bone cell (TGF-β) to osteoinduction (BMP).[6] It is believed that local bone growth factor act in an autocrine or paracrine manner on regional osteoblast and osteoclast.[34]

Adding PDGF or TGF-β to wounds has been shown to stimulate bone, periodontal and skin healing.[8,12,35] PDGF is the first growth factor in the wound, and synthesized by platelets, macrophages, monocytes, and endothelial cells. It stimulates mitogenic activity, angiogenesis, up-regulation of other growth factor and cells, and collagen synthesis.[11,13,17,33-34,36] PDGF acts synergistically with progression growth factors such as the IGF-I.[33] TGF-β is a member of a large family of biologically active protein hormones. It was synthesized by chondrocytes and osteoblast. It stimulates mesenchymal cell growth and differentiation and leads to a rapid deposition of bone matrix. TGF-β can sustain long-term healing and bone regeneration, including bone remodeling of a maturing bone graft.[11,13,17,33-34,36]

Lynch *et al.*[8] demonstrated that PDGF/IGF-I combination could significantly enhance the formation of the new bone and cementum during the early phase of healing. Histologic analysis of biopsies revealed a significant ($p < .01$) increase in test sites compared to controls. Mundy[37] examined the markedly stimulated effects of TGF-β on woven bone formation in the calvaria of mice by the fifth day.

Application of growth factors to implant

Bone response to the implant treated with growth factor has been one of interesting topics for promising fast healing. A few reports used the growth factors as stimulators of implant fixation and bone regeneration.[32,38-49]

Some reported the effects of PDGF alone[40] or PDGF/IGF-I combination[38-39,41,42] on bone regeneration associated with implant. Stefani *et al.*[38] evaluated the effect of PDGF and IGF-I combination associated with implants placed into extraction sockets for different experimental periods in dog. They reported a greater bone-implant contact in test than in control at 3 weeks, but no significant differences in bone area. Lynch *et al.*[39] investigated the PDGF/IGF-I combination effect on press fit implant in dogs and suggested PDGF/IGF-I combination application may improve the prognosis of implants placed into spongy bone which has large marrow spaces and lack of bone in close proximity to the implant, by stimulating the formation of a more dense trabecular bone during the early phase of healing. Nash *et al.*[40] evaluated the effect of PDGF on fracture healing in rabbits using 3-point bending and radiographic methods. They showed higher callus density and volume at 2 weeks and 4 weeks around the treated osteotomies and equivalent in strength to the non operated upon contralateral tibia after 4 weeks. Becker *et al.*[41] compared bone promotion around implants augmented with ePTFE membranes alone or in combination with demineralized freeze-dried bone allograft(DFDBA) or the combination of PDGF/IGF-I in dog. They showed

approximately 2-fold increase in the percentage of bone to implant contact and highest bone density in the dehiscence defects treated with ePTFE plus PDGF/IGF-I after 18 weeks. Nociti *et al.*[42] demonstrated that the presence of a combination of PDGF and IGF-I in the gap created between the implant and bone surface may positively contribute to bone healing process. This was in accordance with Meraw *et al.*' s study.[32]

TGF-β also showed great bone regeneration capacity. Rutherford *et al.*[43] reported the osteogenic protein(TGF-β7) device induced new bone formation in the fresh tooth sockets of monkey within 3 weeks regardless of presence of implants. Lind and coworkers[44-45] investigated the potential of rhTGF-β1 adsorbed onto weight-loaded tricalcium phosphate coated implants in dog. In the gap of test group, bone-implant contact and bone volume were increased significantly.

However, some concluded no significant effect of TGF-β on bone formation. Lind *et al.*[46] reported, while gap healing and shear strength was increased in the study of rhTGF-β adsorbed HA coated implant inserted into bone of 2mm gap, it was not statistically different. Similar result was shown in Cook *et al.*' s study.[47] They used rhOP-1(TGF-β7) in fresh extraction socket of dog and observed at 12 weeks increased osseointegration and new bone formation but it was not significant different.

Platelet-rich plasma (PRP)

PRP is made with the newly developed technique concentrating platelets 3-fold or more.[11,17,22] Through centrifugation of withdrawn blood from the patient, the platelets are consequently sequestered and concentrated in a small volume of plasma to form platelet-rich plasma.[10-11,13,15,18-19] Because platelet is one of the highest concentrations of PDGF and TGF-β in the body[12], concentrated platelets function as the source of growth factor in addition to hemostatic control effect. This PRP is plasma fraction, so it contains not only platelets but also abundant fibrinogen, clotting factors, and cell adhesion molecules like fibronectin, contributing to formation of osteoconductive matrix and cell attachment.[9-10,13,50]

Therefore, activated PRP used for this study consists of two main components, fibrin glue from plasma and platelet concentrates.[13] Fibrin glue are formed from conversion of fibrinogen to fibrin by the action of thrombin. This fibrin network acts as a glue and a scaffold. Thrombin also causes the platelets to degranulate and release PDGF and TGF-β contained in their α granules. This procedure is the initiation of bone regeneration in the graft. PDGF and TGF-β were reported to be approximately equal amount in the PRP gels.[14]

PRP can be used to the particulate graft or soft tissue graft, and in addition, can be used to form a membrane-type gel.[19,21]

Many kinds of PRP preparation technique have been introduced, but they have almost same principles except whether bovine thrombin was used or not[14,23] and commercial fibrin glue (Tissel) was used or not.[19,27]

Application of PRP in the oral and maxillofacial region

There are very few studies about PRP application. In these studies, most of authors, but not all[24,29], reported increased bone regeneration both in the rate and degree. Previous reports have shown the effect of PRP on bone defect[9-11,24], periodontal defect[18], and pre-implantation site preparation such as filling of extraction socket[15,23], ridge augmentation[17,22,24] and sinus lift.[16-17,20,22-23,25] Some authors have tried many kinds of bone substitute such as autogenous bone[9-11,15,22], demineralized freeze-dried bone allograft(DFDBA)[18,24], freeze-dried bone allograft(FDBA)[17], bovine bone mineral[20,23,26,29,31] mixed with PRP as a graft material.

The initial reports about PRP application were

in the case of autogenous bone graft with PRP. In 1994, Tayapongsak *et al.*[9] applied autologous fibrin glue to autogenous bone graft in the mandibular reconstructive surgery and reported about 50% earlier bony incorporation and remodeling with radiographic evaluation. They suggested enhanced consolidation was attributed to interaction of fibronectin, fibrin, factor XIII, etc. Although fibrin adhesive had been used for hemostasis and adhesion of filling materials, healing acceleration through fibrin glue application to autogenous graft was novel finding. Whitman *et al.*[10] stated the difference of components between platelet gel and fibrin glue and introduced the technique for preparation of platelet gel. Marx *et al.*[11] showed the potential of PRP accelerating the rate and degree of bone formation in a cancellous cellular marrow graft reconstructions of mandible. In PRP added graft, radiographic maturation rate was 1.62 to 2.61 times higher and histomorhometric assesment showed a greater bone trabecular area (74.0±11%) than in control group (55.1 ±8%; P= .005) after 6 months healing. They reported the presence and retention of growth factors in the PRP preparation by monoclonal antibody uptake assessment.

Recently, pre-implantation site preparation or periodontal defect treatment by PRP application with various bone substitutes were reported. Anitua[15] filled extraction socket with mixture of PRP and autologous bone and took bone biopsies between 10 and 16 weeks postoperatively. He suggested PRP in the wound improved soft tissue repair and bone regeneration. Rosenberg et al.[16] presented two clinical cases of PRP grafted sinus with alloplast and reported the duration required for graft healing was reduced by virtually 50%. Kassolis *et al.*[17] used PRP in combination with freeze-dried bone allografts(FDBA) for sinus elevation and/or ridge augmentation and reported clinical and histological successful bone regeneration. Obarrio *et al.*[18] used platelet gel in combination with demineralized freeze-dried bone allograft(DFDBA) for the treatment of periodontal osseous defects. Radiographically significant amounts of new bone were visible as early as 2 months postoperatively.

But, Shanaman *et al.*[24] concluded PRP addition did not appear to enhance the quality or quantity of new bone formation over that reported in comparable GBR without PRP. They evaluated the potential of PRP in combination with DFDBA and stated that biochemical interactions between growth factors contained within PRP and the host during wound healing were still not clear, so further controlled studies were required.

In fact, forementioned studies were the results from human application, and some of them could not be evaluated quantitatively. So very recent studies about PRP were tried in the animal model under controlled conditions.[26-31] Kim *et al.*[31] evaluated the animal model, rabbit, with the use of bovine bone mineral (BioOss) mixture with PRP to critical sized bony defect of the calvarium. They showed greater densities in the experimental group and after 8 weeks same mineral densities to host cranial bone. That was in agreement with study of Jang and Kwon[26] used similar bovine bone powder in the rabbit calvaria.

The other experiment in the rabbit was for sinus graft using β-TCP(tricalciumphosphate) in combination with PRP.[38] At 2 and 4 weeks bone volume was higher in the PRP group.

Application of PRP to implant

To date, there are only limited studies on the bone formation effect of PRP associate with implant. Three abstracts and one case report were presented in 2001. The PRP was applied to the situation related with implantation having various modified surfaces, immediate implantation, and sinus lift procedure.

Zechner *et al.*[28] reported increased bone-implant contact and bone volume in the PRP treat-

ed site during the first 6 months in the mandible of minipigs regardless of implant surface structure used. Francesco and Roberto[21] used platelet concentrates and fibrin glue membrane in immediate implantation in human. No bone and no graft material were used. After 40 days noted different degrees of osseointegration were found.

However, Fürst *et al.*[29] demonstrated PRP treated site showed inferior healing around implant during 12 weeks. Bovine hydroxyapatite (BioOss) with PRP were used for the sinus lift of minipigs.[23]

Anitua[23] used PRP/graft mixture for sinus lift with simultaneous implantation.

MATERIAL AND METHODS

Experimental design

Twenty two adult female New Zealand white rabbits, weighing approximately 2.7-3.3 kg, were used for this study. The animals were acclimatized to environment for a month.

Twelve of the 22 animals were used for histomorphometric analysis and ten of the 22 were for mechanical test. Each animal received four implants: two were treated with PRP in one proximal tibia and two were without PRP as control in the contralateral tibia. For histomorphometric analysis, rabbits were divided into four groups according to the healing period. At 1 week, 2 weeks, 4 weeks and 8 weeks postoperatively, each three animals were sacrificed serially and the amount and rate of bone formation around dental implant were examined. For mechanical test, rabbits were divided into two groups and removal torque values were evaluated at 4 weeks and 10 weeks after implant placement.

Implant and PRP preparation

Screw-shaped, commercially pure titanium implants (Neoplant, Neobiotech, Seoul, Korea), 7mm in length and 3.75mm in diameter were used in this study. In total, 88 fixtures were used.

PRP was developed from autologous blood of the rabbit. After anesthesia the 6ml of blood were withdrawn from each animal through heart puncture and transferred to centrifuge tube containing 1 ml of ACD-A(acid citrate dextrose) solution (CTG, Korea United, Korea) for anticoagulation. By means of a centrifuge (Placon, Oscotec, Chonan, Korea), the blood was centrifuged at 2000G for 3 minutes and finally separated into three basic component layers due to its gradient density: Platelet poor plasma(PPP) at the top, platelet-rich plasma(PRP) in the middle and red blood cells(RBCs) at the lowest level (Fig. 1). PPP and PRP layers including small amount of upper RBCs were collected to new tube and centrifuged again for precise separation at 5000G for 5 minutes. After a second centrifugation, the final fractions were developed: PPP was formed as a top level looks clear yellow, which contained poor platelets; PRP, which had concentrated platelets, was formed beyond the RBCs layer. This PRP layer, approximately 10% of whole withdrawn blood, was collected with a pippette in the bowl. The PRP was stored at room temperature until the implant bed was prepared.

Surgical procedure

Animals were premedicated with an intravenous injection of 2ml of amoxicillin with clavulanic acid (30mg/kg, Augmentin, Ilsung, Korea) prophylactically prior to surgery.

Surgical anesthesia was obtained by intramuscular injection of ketamine hydrochloride (30mg/kg, Ketamine, Yuhan, Korea) and 2% xylazine hydrochloride (0.15ml/kg, Rompun, Beyel, Korea). Anesthesia was supplemented with local infiltration of 2% Lidocaine Hcl (1:80,000 epinephrine, Gwangmyung, Korea).

The skin of the hind legs was clipped, disinfected with povidone iodine. Skin incision was made along the proximal one third of the tibia. After full thickness flap reflection, two holes about 1 cm

apart, were drilled with an internally irrigated equipment on the mesiolateral surface of proximal tibia. Conventional drilling procedures suggested by Brånemark were performed. After final low-speed tapping, bone bed were ready to be implanted.

Test implants were immersed into prepared PRP concentrates, and coagulated by subsequent adding a mixture of 0.1ml of 10% calcium chloride (Calmia, Korea United, Korea) and 1000units of bovine thrombin (Dirabine, Korea United, Korea) just before placement. Within a few seconds, the contents assumed a gel-like consistency as the thrombin reacts with the fibrinogen, resulting in the formation of fibrin (Fig. 2). The fibrin formation of the PRP process serves as a glue, so direct adsorption of PRP onto the surface of implants was achieved (Fig. 3). The PRP gel formed around implant was cut to mass carefully, leaving sufficient volume in situ coagulated onto implant surface.

Harvested gel mass was placed into prepared holes of implant bed before PRP-treated implant insertion.

The contralateral side was prepared as same methods as described previously except that has no PRP-related procedure. Two dental implants were placed on each side of the tibia of each an-

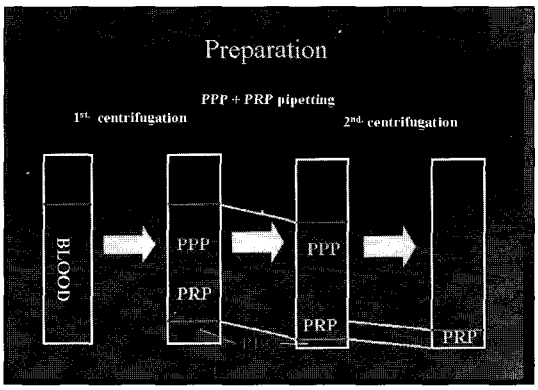

Fig. 1. Procedures for platelet-rich plasma preparation.

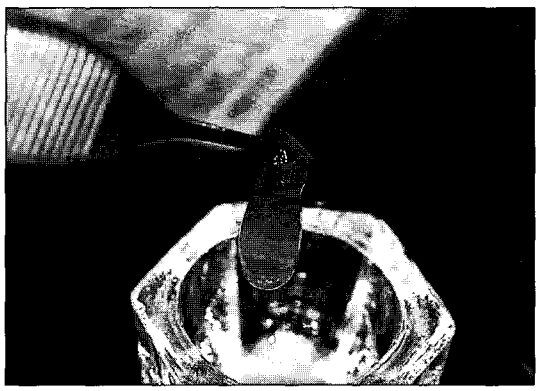

Fig. 2. Platelet gel activated with thrombin.

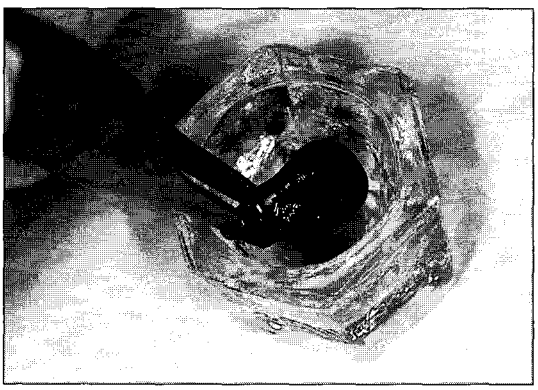

Fig. 3. Platelet gel adsorbed onto implant.

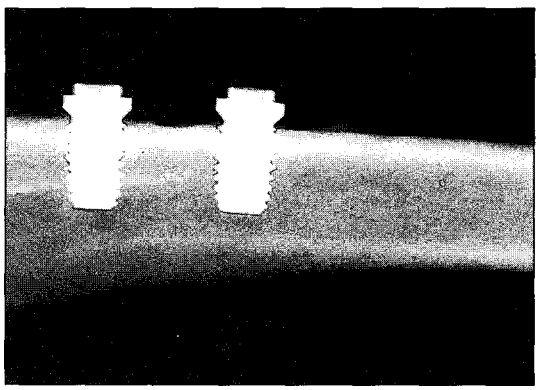

Fig. 4. Radiographic view taken 4 weeks after implant insertion in the rabbit tibia.

imal.

For the experimental groups of removal torque test, implant insertion was cautioned not to be engaged bicortically (Fig. 4). One thread was left visible above the cortex.

The incision was closed in layers with interrupted suture of the synthetic absorption material. The limbs were bandaged to avoid wound dehiscence.

All procedure was performed with an aseptic technique. Postoperatively animals received conventional antibiotic therapy(amoxicillin with clavulanic acid, I.M.) for two days.

Bone labeling procedure

To determine the activity period and rate of bone formation, fluorochrome was administered at several different times throughout the experiment according to the schedule (Table 1): Tetracycline HCL (TC, 20mg/kg, Sigma, Sigma chemical, St.Louis, USA) dissolved in saline, Calcein green (CAL, 15mg/kg, Sigma, Sigma chemical, St.Louis, USA) and Alizarin red S (ARS, 20mg/kg, Sigma, Sigma chemical, St.Louis, USA) dissolved in a sodium bicarbonate solution (20g/l) were given in turn intramuscularly.

Preparation of the specimens

After healing, the animals were anesthetized as was previously described and sacrificed in the CO_2 chamber.

The tibiae were harvested and sectioned. Block sections of implants with surrounding bone were fixed in 10% neutral buffered formalin solution at 4℃ for a week. The specimens were prepared according to the method of Donath et al.[52] The specimens were dehydrated in a graded ethyl alcohol series, then infiltrated and embedded in methyl methacrylate (Technovit 7200 VLC, Exakt, Germany). The specimens affixed onto acrylic slides were sectioned along the longitudinal axis of the implant on a low-speed diamond band saw (Exakt, Germany) and ground to 30㎛-thickness for fluorescent microscopy and histomorphometric analysis with carborundum papers ranging from # 800 to 1200 using a microgrinding system (Exakt, Germany).

Fluorescent and polarized microscopy

To study the time of new bone formation, ground sections were examined under a fluorescence microscope before histologic staining. Labeling patterns of each period were observed. In 4- and 8-week specimens, lamination pattern was also investigated with polarized microscopy.

Histomorphometric examination

After fluorescent examination, the specimens were surface stained with 1% toluidine blue for 20 minutes and used for blinded quantitative histomorphometric evaluation of bone growth. By light microscopy at a magnification of ×100 interfaced with computer-assisted image analysis program (Image-Pro Plus, USA), the percentage of bone to implant contact (BIC) and bone volume within threads were calculated in the constant region throughout the examination. For the com-

Table Ⅰ. Labeling schedule.

GROUP	WEEKS						
	BASELINE	1	2	3	4	6	8
1-WEEK	-	SACRIFICE					
2-WEEK	TC	CAL	SACRIFICE				
4-WEEK	TC	-	CAL	ARS	SACRIFICE		
8-WEEK	-	-	TC	-	CAL	ARS	SACRIFICE

TC : Tetracycline, CAL: Calcein, ARS: Alizarin red S

parison of approximately the same dimension of threads of fixtures during the healing, histomorphometric measurements were restricted to the total upper cortical bone area and into the limits of three grooves (thread bottoms) of endosteal area in the upper marrow region on the basis of dimension of 1-week specimen. Because this endosteal area was filled later with new lamella bone grown downward along the implant surface from upper cortical bone, separate measurements in the cortical and/or marrow region of each periods were not inadequate. Lower part and cutting edge portion of implant in the marrow cavity were not considered to count due to irregular number of threads and different proximity to lower cortical bone.

Removal torque test

Removal torque was measured with a torque gauge (Tonichi 15-BTG, Japan). The torque value necessary to unscrew the implant reflects the shear strength of the bone/implant interface.

One rabbit was died 1 week postoperatively, so removal torques of nine rabbits were evaluated. Torque was investigated at 4 weeks (five of the 10 rabbits) and 10 weeks (four of the 10 rabbits) postoperatively.

The animals were anesthetized and sacrificed as was previously described. After exposure of tibial area, bone formed on the top of the implants were removed carefully.

To attach a torque gauge to the implants, fixture mount was connected securely and then countertorqued to failure. The values to loosen the implants were dictated by dial memory gauge.

Statistical analysis

Data are summarized as mean ± SD. For statistical evaluation, the data were analyzed using a nonparametric Mann-Whitney rank test. Significant differences were considered at $P < .05$.

RESULTS

In this study bone regeneration was evaluated histologically, morphometrically and mechanically.

Fluorescent and polarized microscopy

Fluorescent microscopy of the specimen showed different color lines, suggesting continuing osteogenesis. These labels revealed extensive bone formation at and near the implant surface, especially in the endocortical area of marrow cavity. The fluorochrome label appeared according to a injection order of Table Ⅰ. One-week group had no fluorochrome given. Two(2-week specimen) or three(4- and 8-week specimens) different color lines were arranged with parallel lamination patterns, which corresponded to the layers of newly formed bone on the day of fluorochrome injection. Tetracycline(TC), Calcein green(CAL), and Alizarin red S(ARS) fluoresced yellow, green, and red respectively at a different wavelength with ultraviolet(UV) illumination.

Bone apposition was progressed from the surface of cortical bone outward in the periosteal layer and inward in the endosteal layer. At the bone-implant interface the newly formed bone extended from the surface of existing bone to the titanium implant surface.

1) 2-week specimen

There was no labeling in the nearly all area of cortical bone close to the implant. At the endosteal surface of cortical bone, yellow line of TC (formation at baseline) uptake appeared. Green line of CAL (formation at the first week) uptake was observed in the marrow cavity and periosteal surface. In the marrow cavity, green line was entrapped with trabecular bone formed extensively after 1 week. That revealed the differences of the amount of newly formed bone between at 1 week and 2 weeks after implant insertion. Within threads, bone label appeared more abundant

and nearer to the implant surfaces and was observed in larger areas of the marrow cavity in the PRP group than in the control group (Fig. 5,6).

2) 4-week specimen

Yellow line of TC (formation at baseline) was restricted to endosteal surface, too. In the marrow cavity, abundant woven bone was resorbed as a procedure of normal remodeling. Bone filling was limited within threads near the endosteal surface, but label was scattered along the implant surface. At the endosteal portion near the implant surface in the marrow cavity, there was specific pattern of bone labeling. Thin red line of ARS was approaching the implant surface and growing downward.

In the PRP specimens, active CAL-labeled bone (formation at the second week) occupied most of area within threads and was in contact with implant surface. In some areas, erosion cavity was observed in the CAL-labeled bone and relined with thin ARS label. Relatively little ARS label (formation at the third week) was found in the gap.

In the control specimens, ARS-labeled bone and bone formed after two weeks occupied larger areas than in the PRP specimens. Erosion cavity was observed in the ARS-labeled bone or bone formed thereafter.

3) 8-week specimen

At the site of newly formed cortical bone in the marrow cavity, active uptake of bone label was observed. The most of bone within threads were occupied with fluorescent labels of TC (formation at the second week).

In both PRP and control specimens of thread bottoms, bone was extending to the implant surface. However, there were differences between them. In the PRP specimen, lamination patterns of CAL(formation at the fourth week) and ARS(formation at the sixth week) were lined orderly and distances between lines were short (Fig. 7). On the contrary, in the control specimen many portions of the green labels of CAL were replaced by the red labels of ARS within threads and moreover, the area of bone marked with ARS and thereafter (formation at from the sixth week to the eighth week) were grater than in the PRP specimen (Fig. 8). In the marrow cavity, downward grown cortical bone outside the thread edge showed different label distance between PRP and control specimens, too. In the PRP specimens, distance between ARS label and CAL label was not so long as that in the control specimens.

Polarized microscopy of 8-week specimens supported this chronologic pattern of bone formation. Parallel lamination layers with isotropic pattern, reflecting the packing of collagen fibrils formed later were observed around preformed bone and their areas were larger in the control than in the PRP specimens (Fig. 9, 10).

Histological examination

Specimens were examined using a light microscope at a magnification $\times 100$ and $\times 200$.

Woven bone appeared heavily stained by toluidine blue and could easily be observed. On the contrary, the mature bone was stained more lightly.

1) 1-week specimen

In the cortical region of PRP specimen, bone fragments came from drilling procedure were found in the gap between the implant and surrounding bone. Of which, some were added with woven bone. Red blood cells were seen in the gap. Although a little gap was filled with violet stained osteoid like woven bone, only small part of the implant was contact with bone in the cortical region (Fig. 11b). In the marrow cavity, a trabecular bone formation was evident from the endosteal surface to cavity, especially at upper and lower endocortical region near the implant. A lattice of trabecular bone was approaching the implant but very

little bone was slight touch in contact. The bone was loosely organized woven bone (Fig. 11c).

In the cortical region of control specimens, the gap was filled not with any osteoid but with red blood cells (Fig. 12b). In the marrow cavity, trabecular bone was found at the endosteal surface near the implant, but the range and extent of bone volume along the implant was low compared to PRP treated region (Fig. 12c).

2) 2-week specimen

Periosteal woven bone was markedly increased compared to 1-week specimen.

In the cortical bone of PRP specimens, the gap was filled with woven bone so much that little space was remained (Fig. 13b). At the medullary region, extensive bone formation was seen inside the cavity along the implant surface and woven bone was in direct contact with the surface in many areas of threads. The threads were almost filled with newly formed bone (Fig. 13c).

In the cortical bone of control, gap filling was less than that in the PRP group (Fig. 14b), and the periosteal bone formation was less, too. In the marrow cavity, woven bone was limited to endosteal region near the upper and lower portion of implant and stained more heavily like that seen in PRP specimens of 1-week (Fig. 14c).

3) 4-week specimen

Periosteal bone was still increasing along the implant neck, but most of bone were woven.

In the cortical region of both PRP and control specimen, the gap was almost filled with dense woven bone similar to lamella bone. Bone area was larger in the PRP specimen. Intracortical remodeling was evident, especially in the PRP specimens (Fig. 15a, 16a).

In the marrow cavity of both specimens, trabecular bone was growing downward. Woven bone had changed to mature bone with lamella structure and the bone volume of endocortical area was decreased thinly (Fig. 15b, 16b). Woven bone which had filled the thread was remodeled. Cavity was composed mainly of fat cells and some marrow cells.

Small area of bone with lamella pattern was seen like a islet in the bottom or edge of the thread along the implant surface.

4) 8-week specimen

Periosteal bone was matured to lamella bone and was being remodeled. In the cortical region of both PRP and control specimens, most of threads were filled with lamella bone, and cortical bone from the endosteal surface was growing downward more than that of 4- week specimen (Fig. 17a, 18a). The remodeling near the implant surface was seen as the form of erosion cavity and newly formed, heavily stained bone (Fig. 17b, 18b). In the marrow cavity of both specimens, areas of bone coverage were increased compared to 4-week specimens.

Histomorphometric examination

Means and standard deviations were calculated for each measured parameter. In the control region, bone implant contact and bone volume showed an increase with time. In the PRP treated region, however, bone implant contact and bone volume rapidly increased at early healing period and maintained thereafter (Table Ⅱ, Ⅲ, Fig. 19, 20).

PRP group showed significantly higher bone volume within threads compared to control group at 2 weeks ($70.30 \pm 4.96\%$ vs. $50.68 \pm 6.33\%$; $P < .01$) and 4 weeks ($82.59 \pm 5.94\%$ vs. $72.94 \pm 4.57\%$; $P < .05$)(Table Ⅱ, Fig. 19). There were no significant differences between them at 1 week and 8 weeks. PRP group at 1,2 and 4 weeks revealed similar degree of bone volume formation comparable to control group at 2, 4 and 8 weeks, respectively.

On the other hand, while PRP group showed

Table Ⅱ. Bone volume within threads (%)

GROUP	PERIOD			
	1 WEEK	2 WEEKS	4 WEEKS	8 WEEKS
PRP	48.17±4.39	70.30±4.96**	82.59±5.94*	78.00±7.49
CONTROL	48.84±8.85	50.68±6.33	72.94±4.57	82.68±6.84

Mean ± SD. *: P < .05 **: P < .01, as compared with control at each period.

Table Ⅲ. Bone-implant contact (%)

GROUP	PERIOD			
	1 WEEK	2 WEEKS	4 WEEKS	8 WEEKS
PRP	26.13±4.04	47.37±8.09	38.44±8.71	42.43±9.24
CONTROL	28.34±14.70	33.16±13.47	47.34±4.14	48.4±8.03

Mean ± SD. No significant differences as compared with control at each period at P < .05.

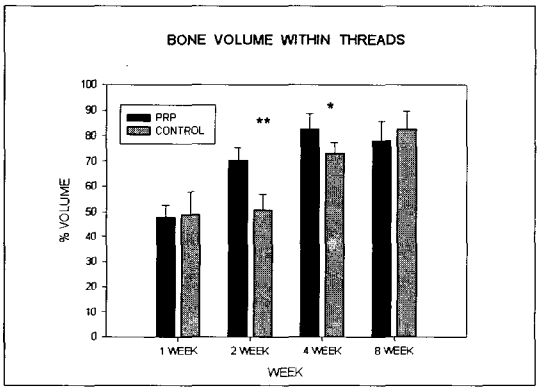

Fig. 19. Bone volume within threads (%).
*: P < .05 **: P < .01

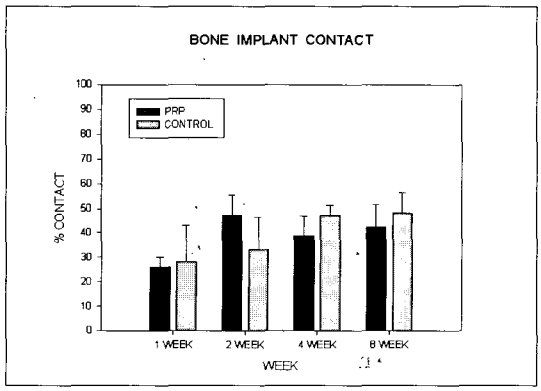

Fig. 20. Bone-implant contact (%).

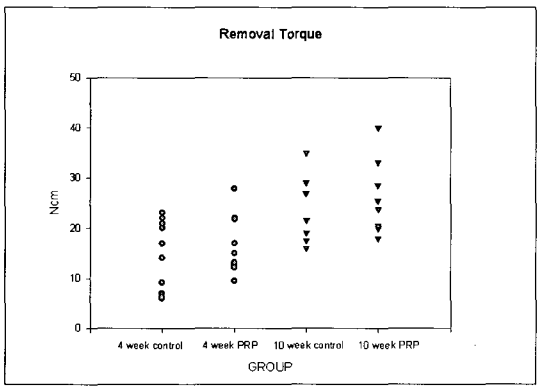

Fig. 21. Removal torque (Ncm).

Table Ⅳ. Removal torque values (Ncm)

GROUP	PERIOD	
	4 WEEKS	10 WEEKS
PRP	16.3±5.8	26.2±7.4
CONTROL	14.5±6.9	23.0±6.6

Mean ± SD. No significant differences as compared with control at each period at P < .05.

higher bone-implant contact (47.37±8.09%) than control group (33.16±13.47%) at 2 weeks, it was not statistically significant (P = .082)(Table 3, Fig. 20). With a regard to bone implant contact, there were no significant differences between PRP group and control group for any experimental period.

Removal torque test

The mean peak removal torque was higher in the PRP group than in the control group at both periods. But it was not statistically significant (P > .05) (Table Ⅳ, Fig. 21).

DISCUSSION

Platelet-rich plasma (PRP) has been used to reconstructive surgery and site preparation in relation to the implant placement in the oral and maxillofacial region. In this study, the results were consistent with previous studies to a certain extent. The results of this study provide evidence that PRP, even without bone substitute as a scaffold, could induce bone formation around implant more and faster in the rabbit tibiae.

By virtue of growth factors contained in α granule of concentrated platelets, PRP has been known to accelerate the rate of bone regeneration as well as the degree of bone density.[11,13] They influenced the bone regeneration in the first few weeks, however, the lifetime of growth factor is very short. Lind *et al.*[44] observed 90% of adsorbed radiolabelled rhTGF-β1 was released within four hours of incubation in serum. Lynch *et al.*[8] also reported clearing the 96% of the radiolabelled proteins by 96 hours and no detection 2 weeks after application. The life span of a platelet in a wound and the period of the direct influence of its growth factor is less than 5 days.[11] In spite of short diminishing time of growth factor it seems to stimulate a cascade of wound healing events that continues even in their absence.[8] The extension of healing and bone regeneration activity is accomplished by two mechanisms. The first is the increase and activation of marrow stem cells into osteoblasts, which then secrete TGF-β and IGF into osteoid matrix. The second and more dominant mechanism seems to be through the chemotaxis and activation of macrophages, which replace the platelet as the primary source of growth factor.[11,13]

Studies of single growth factor inducing bone regeneration have been reported very much.[8,35,38-49] They were consistent with the results of PRP studies in bone regeneration during early healing period, but there are some differences between them. For example, single recombinant growth factor has a single generation pathway. On the other hand, PRP contains many growth factors and proteins that modulate and upregulate one growth factor' s function in the presence of second or third growth factor.[13] Indeed, PRP has many advantages compared to recombinant growth factors. Above all, it doesn' t cost due to autologousity. Although recombination technique can produce larger quantities under controlled laboratory conditions without hazard[8], recombinant growth factors have difficulty in availability for clinical use due to its high purification cost.[18]

Easy manipulation is another advantage of PRP. PRP technique allows to concentrate naturally occurring growth factors for immediate clinical use in need prior to surgery.[12-13] It takes very short time to be produced, and moreover, through the activation with calcium chloride and thrombin PRP has a gel-like consistency which helps to bind graft material together. That facilitates clinical handling of the graft material.[9-10] It also contributes to hemostasis. This gel formation is due to the fibrin network, which acts as a medium for compacting grafts, a osteoconductive material, a graft vehicle, and also a barrier.[9-11]

Although many studies using growth factor

with implant were published, there were no reports investigating mechanisms of PRP associated with implant. Previous studies that have reported PRP use were for either maxillofacial surgery like recovering bony defect or pre-implantation site preparation.

In this regard the present experiment had differences with previous studies. One is to investigate the effect of PRP on bone response associated with implant material under controlled conditions, the other is to use PRP without bone substitute as a scaffold.

To compare the bone formation around the implant during the healing, the time-labeling method was used. Mineralizing process observed by fluorescent microscopy was attributed to fluorochrome activity to bone. These fluorochrome binds avidly with bone mineral but does not affect the proliferation and metabolic activity of osteoblastic cells.[53]

The formed fluorescent labels could be evaluated under fluorescent microscope for assessment of mineral apposition rate. This approach helps to understand the continuous bone formation procedure instead of transient observation at a specific time by histomorphometric analysis. In the fluorescent microscopy of 2-week specimen, bone formed around the implant at 1 week and 2 weeks after implant insertion were more and nearer to the surface in the PRP group. Bone formation rate at each period was different between two groups. Bone labels of 8-week specimen revealed more bone formation before four weeks in the PRP treated site and little bone formation after four weeks. On the contrary, bone formation after four weeks in the control group was greater than that in the PRP group. Continuous increasing trend of bone with time was observed in the control group. That means that although similar amounts of bone were formed in two groups at 8 weeks, they could have different formation period each other according to whether PRP was applied or not.

In the histologic observation, the range of woven bone along the implant in the marrow cavity were greater in the PRP group than in the control of both 1- and 2-week specimens. And some osteoid was found in the cortical gap of PRP treated region of 1-week specimen. It was in contrast to previous study. Sennerby *et al.*[54] reported that new bone formation in the cortical part appeared after 14 days, though bone formation in the marrow before 7 days.[54-56]

In the histomorphometric analysis, bone volume within thread was increased earlier in the PRP specimen and so it was comparable to that in one-step later period of control group. That reflected the accelerated bone formation in the PRP treated site. But significant differences of bone-implant contact were not detected between PRP and control group for any experimental period. It was somewhat unexpected and contrary to hypothesis that growth factors in PRP could enhance bone-implant contact as well as bone volume earlier than control. The probable reason was that measurements were performed within limited specimens of 2-D(dimensional) plane instead of 3-D(dimensional) structure, because bone appeared in one plane was cross-section of extension from other plane. This was supported by some authors.[57-58] Wigianto *et al.*[57] concluded the bone-implant contact rate depended on the cutting position and direction in the specimen. Ettinger *et al.*[58] insisted one must think of the percentage of bone-implant relationship as constantly changing, depending on the dynamics of the bone in contact with the implant at that point in time. Furthermore, some studies revealed no correlation between bone-implant contact and bone volume in chronological bone regeneration of growth factor treated site.[38-39,42] The other reasons to be considered were the possibility of collapse of the platelet gel on the implant surface placed in the medullary cavity during insertion procedure and insufficient spread-

ing of inserted gel due to loose marrow tissue.

One assumption made for PRP around implant was remarkable enhancement of bone regeneration, especially in the cancellous region[39], because receptors for PDGF and TGF-β had been found in cancellous cellular marrow.[11,15] These findings identified the presence of abundant marrow stem cells and osteoprogenitor cells within cancellous marrow capable of responding to the increased PDGF and TGF-β in the PRP preparation.[4,11] Moreover spongy bone normally exceeds compact bone in new bone formation activity[59] which is a prerequisite for secondary fixation.[4,39,56] This was supported by other researcher.[60] In this study, bone volume of PRP group was significantly increased 2 weeks and 4 weeks after implant placement. Although bone-implant contact was not increased so much as described earlier, volume increase might be attributable to the receptors for growth factors in the marrow cavity. Because the fixation of implant is dependent on the state of the host bone and its healing capacity, PRP-induced host bone change could affects the long-term clinical success.[4,56]

According to recent studies, the failure of osseointegration is not attributed to loading itself but to excessive micromotion induced by loading.[61-65] Therefore if the micromotion over the threshold could be avoided, unloading period may be unnecessary and/or at least shortened. A rapid and more bone ongrowth to implant by PRP can eliminate the risk of micromotion and then, be helpful to early loading of implants. Removal torque test was performed in this point of view, because it can evaluate the quality of implant osseointegration. However, contrary to hypothesis, removal torque values were not significantly different between PRP group and control group. The removal torque reflects both bone thickness and bone quality but is limited to cortical bone surrounding the implant not the total bone.[66-68] Therefore the result demonstrates no mechanical improvement was induced in the cortical bone of PRP treated group in spite of early osteoid formation in the gap observed in the histologic finding. Some researchers have shown a correlation between histomorphometry and mechanical parameters in growth factor group.[40,46,67,69-70] But the result of no improvement cannot be ascribed only to its no significance in the bone-implant contact(BIC), since BIC may not be correlated to removal torque.[66] For example, thin bony coating formed on the implant surface at later healing period is not engaged in load transfer though it contributes to increase of bone-implant contact ratio. With respect to osteoporosis, although osteoprotic bone showed similar bone-implant contact percentage,[72-74] it has lower removal torque values[75] and thinner cortical bone thickness.[76]

In addition, mechanical test at early healing period might be a more crude method than histomorphometry, and the modest increase or decrease in bone ongrowth be too small to affect significantly the mechanical fixation.[44-46,71] Therefore similar removal torque values of two groups were rather due to its evaluation time. Woven bone cannot resist functional load.[59] At 4 weeks histomorphometric study indicates little part of woven bone was being remodeled to lamella bone in some surfaces of implant. According to Roberts *et al.*[55], in rabbits it takes 6 weeks for the woven bone to be replaced by lamella bone with adequate strength for load bearing. Even at 10 weeks difference of maturation was too little to reflect significant values because it was only after one sigma(remodeling cycle). In spite of these results, however, woven bone was known to serve an implant stabilization role in postoperative healing of endosseous implants.[56,59]

On the basis of this fact, slight increase of bone volume and strength induced by PRP are considered to contribute to secondary stability avoiding micromotion, though it cannot resist to early functional load.

The rationale for using bone grafts in the growth factor application is for providing a space as a scaffold and acting as a carrier of growth factor.[77] Nowadays, different bone graft materials with PRP has been tried for bone augmentation but the responses of bone regeneration to the PRP application site were different each other according to the bone substitute material. The autogenous bone grafted sites showed increased bone formation in all studies[9-11,15,22] due to cells and growth factors included in the autogenous bone. In contrast, in some bovine hydroxyapatite(BioOss) substitue[29,78] or DFDBA augmentation sites[24], no significant effects were demonstrated. The reason of these conflicting results were not clear but, if it was due to no or limitted role of a osteoconductive or osteogenic material, platelet gel application alone by adsorption onto implant and insertion into bone bed could be suggested to induce bone formation around implant regardless of bone substitute.

The goal of biochemical surface modification is to immobilize proteins, enzymes, or peptides on biomaterials for the purpose of inducing specific cell and tissue response or, in other words, to control the tissue-implant interface with molecules delivered directly to the surface.[6] To elongate the effect of biomolecules, delivery system of growth factor has been considered many ways.[79-83] Of which, the simplest way to deliver biomolecules to the implant interface is dipping the implant in a solution of protein before placing it.[6]

Dipping technique is usually adopted to application of growth factors[40,44,46] in spite of disadvantage of difficulties in adsorption and release control over the delivery.[6] The exact amount of recombinant material delivered to each site could not be measured or controlled and may have influenced the results. The poor handling of characteristics resulted in significant loss of material from the site at the time of surgery.[47] But in case of PRP, dipping gelation seems not so critical as recombinant growth factors to get effective threshold dose. In this study PRP adsorption onto implant surface was attributed to its fibrin action.

Generally, the event that occurs almost immediately upon implantation materials is adsorption of proteins.[84] These proteins come first from blood and tissue fluid at the wound site and later from cellular activity in the interfacial region.[6] The nature of this film deposition on biomaterial can be a major determinant of the host response.[50] Some concluded early blood cell/interface interactions play a key role in the osteoconduction of bone.[51,84] Fibrinogen and fibronectin etc. contained in plasma and growth factors released from platelet granules might be expected to act in this film of the implant surface with surrounding bone cell. Really, the interface of implant with surrounding bone comprised predominantly fibrin and red blood cells[51,85], therefore adsorbed fibrin gel to the implant in this study could induce this interfacial environment early and effectively.

Healing involves the replacement of the initial clot by granulation tissue.[86] So the surface of implant might affects the healing rate. Previous investigation proved that rough implant surface promotes faster direct contact with bone than do smooth-surfaced implants.[4] Although machined surface implant was used for the reaction with PRP gel in this study, with regard to platelet and fibrin clot aggregation, implant surface characteristics could be a important factor to be considered.[51,85]

Majority of previous studies about PRP were application to human. As a statement of Shanaman *et al.*[24], however, evaluation in human has limitations in assessing values and that is because why the controlled studies in the aniaml are required. Dog or monkey has been used as the animal model in most of studies of growth factor associated with implants, but to date there was very little information about effective experimental

animal in PRP application. Recently Kim *et al.*[31] introduced the rabbit model and investigated the amount of platelet concentration. They showed average 287% concentration in the rabbit and it was comparable to study of Marx *et al.* in which a 338% concentration was measured in the PRP of human.[11] Three- to ten-fold concentration was also reported in human.[17,22]

One of the problems with this study was no loading environment since that can inhibit tissue differentiation.[87] The other problem could arose from limitation of bone composition of the rabbit tibia. In fact, rabbit has been suggested to useful animal models for preparation of PRP.[31] They have many advantages such as similar ultrastructure and constituents of the platelet, easy manipulation and sufficient volume of blood. Besides, they show survival time of platelet similar to the metabolic bone activity and high cross-reactivity of anti-human antibodies for immunohistochemical studies. However, the rabbit tibia is mainly composed of cortical bone and marrow cell but no cancellous bone.[54] It could cause insufficient spreading of PRP mass inserted into drill hole and deteriorating contact to the implant in the marrow cavity.

But despite of these limitations, results demonstrated that PRP could enhance the rate and degree of bone formation, especially in the marrow cavity and small gap between the implant and surrounding cortical bone, though it has not induced bone maturation enough to resist functional loading. Therefore it might be concluded that PRP can be successfully used to poor bone quality[72-75,88-89](e,g. osteoporotic bone, maxillary spongy bone, bone in elderly individuals) and small gap (developed during immediate implantation) for faster stabilization as previously suggested. And PRP will aid in shortening healing period after implantation.

In this study, PRP application to implant treatment suggested that growth factors can be used conveniently without infection, toxicity or financial problems.

CONCLUSION

Platelet-rich plasma (PRP) was applied to the implant and bone bed without carrier or scaffold material in the rabbit tibia. At various healing periods, bone formation rate and degree around implant were evaluated by histological, histomorphometric and mechanical measurement.

1. In the fluorescent and polarized microscopy, labeling pattern reflected earlier bone formation in the PRP group than in the control group and bone formation rate at each period was different each other.
2. Histomorphometrically, PRP group showed significantly higher bone volume within threads than control group at 2 weeks ($70.30 \pm 4.96\%$ vs. $50.68 \pm 6.33\%$; $P < .01$) and 4 weeks ($82.59 \pm 5.94\%$ vs. $72.94 \pm 4.57\%$; $P < .05$).
3. Histomorphometrically, while PRP group showed higher bone-implant contact ($47.37 \pm 8.09\%$) than control group ($33.16 \pm 13.47\%$) at 2 weeks, there were no significant differences between PRP group and control group for any experimental period ($P > .05$).
4. Removal torque values also showed no significant differences between PRP group and control group at any experimental period ($P > .05$).

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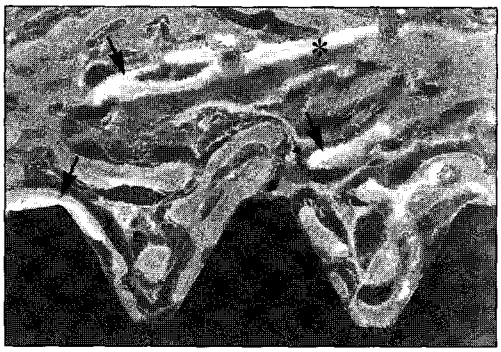


Fig. 5. Fluorescent microscopy of a ground section, showing TC labeling(asterisk) and CAL labeling(arrows) and abundant woven bone in the marrow cavity of PRP group at 2 weeks after implant insertion (original magnification ×100).

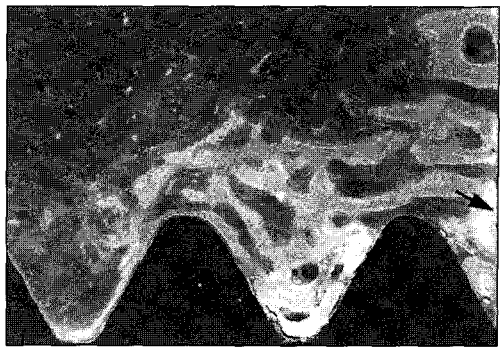


Fig. 6. Fluorescent microscopy of a ground section, showing less labeling(arrow) and woven bone in the marrow cavity of control group at 2 weeks after implant insertion (original magnification ×100).

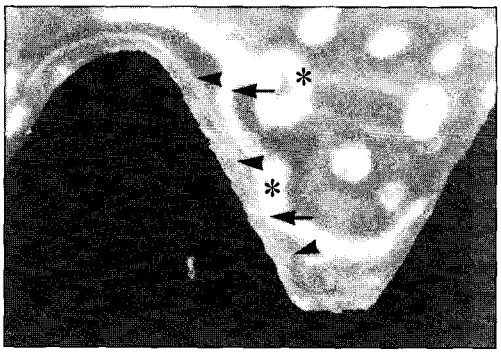


Fig. 7. Fluorescent microscopy of a ground section at the down-grown cortical bone site of PRP group taken 8 weeks after implant insertion (original magnification ×200). TC, asterisks; CAL, arrows; ARS, arrow heads.

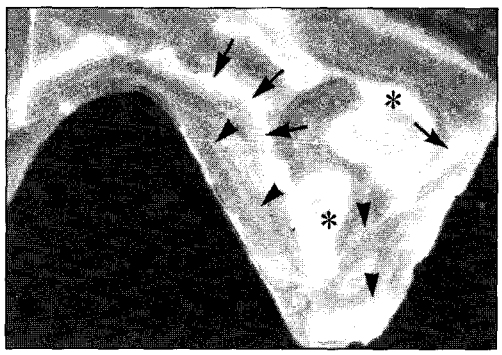


Fig. 8. Fluorescent microscopy of a ground section at the down-grown cortical bone site of control group taken 8 weeks after implant insertion (original magnification ×200). TC, asterisks; CAL, arrows; ARS, arrow heads.

Fig. 9. Polarized microscopy of a ground section taken 8 weeks after implant insertion (original magnification ×200). Thin intermediate bone(asterisks) was shown with isotropic pattern around preformed bone.

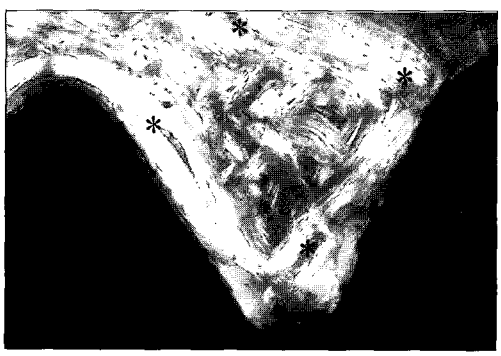
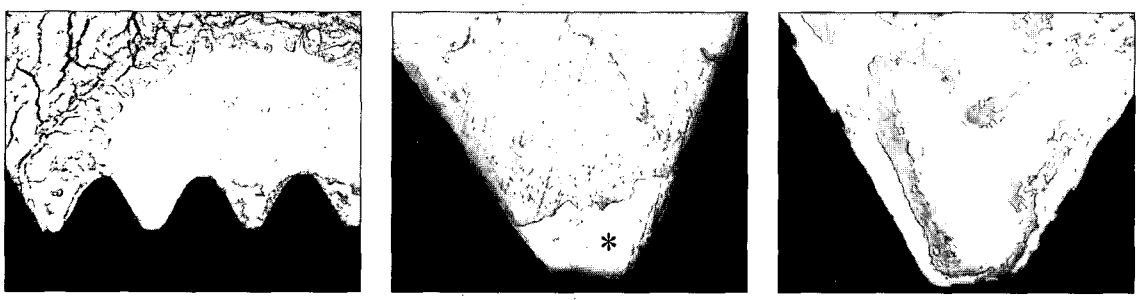


Fig. 10. Polarized microscopy of a ground section taken 8 weeks after implant insertion (original magnification ×200). Thick intermediate bone(asterisks) was shown with isotropic pattern around preformed bone.

(a) (b) (c)

Fig. 11a to c. (a) Histologic findings of a ground section of PRP group at 1 week after implant insertion (toluidine blue stain, original magnification ×40). (b) Gap was filled with osteoid(asterisk) at the cortical bone site (original magnification ×200). (c) Trabecular woven bone was approaching the implant in the marrow cavity (original magnification ×200).

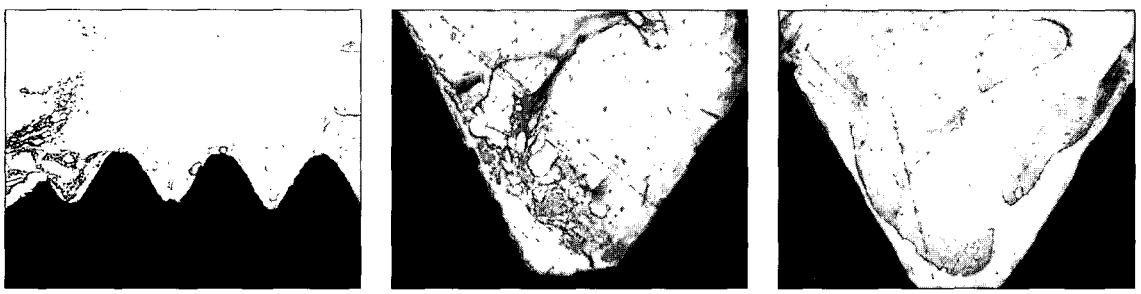


(a) (b) (c)

Fig. 12a to c. (a) Histologic findings of a ground section of control group at 1 week after implant insertion (toluidine blue stain, original magnification ×40). (b) Gap was not filled(asterisk) at the cortical bone site (original magnification ×200). (c) Less woven bone was formed in the marrow cavity (original magnification ×200).

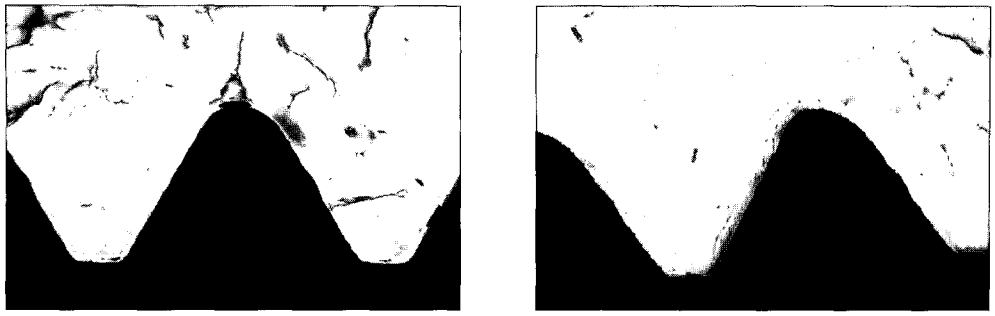
(a) (b) (c)

Fig. 13a to c. (a) Histologic findings of a ground section of PRP group at 2 weeks after implant insertion (toluidine blue stain, original magnification ×40). (b) Large space of the gap was filled with woven bone at the cortical bone site (original magnification ×200). (c) Newly formed bone in the marrow cavity (original magnification ×200).



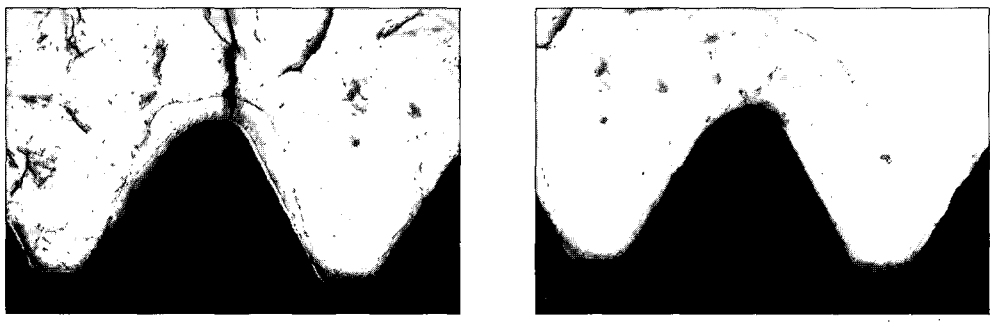
(a) (b) (c)

Fig. 14a to c. (a) Histologic findings of a ground section of control group at 2 weeks after implant insertion (toluidine blue stain, original magnification ×40). (b) The gap was filled partially at the cortical bone site (original magnification ×200). (c) Heavily stained bone in the marrow cavity (original magnification ×200).



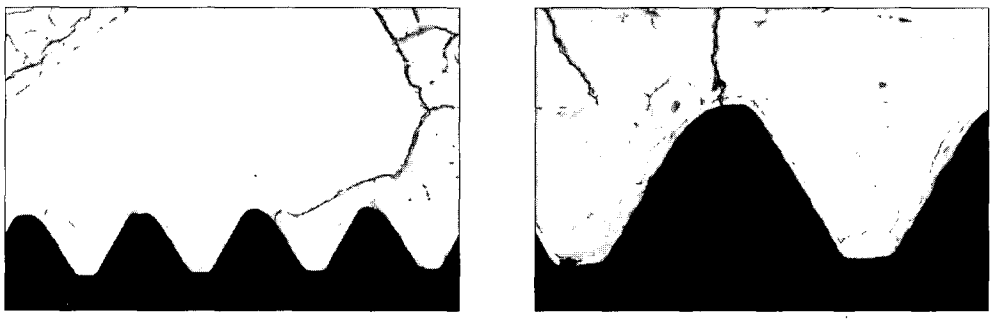
(a) (b)

Fig. 15a and b. (a) Histologic findings of a ground section at the cortical bone site of PRP group at 4 weeks after implant insertion. Intracortical bone remodeling (toluidine blue stain, original magnification ×100). (b) Thinly down-grown cortical bone (original magnification ×100).



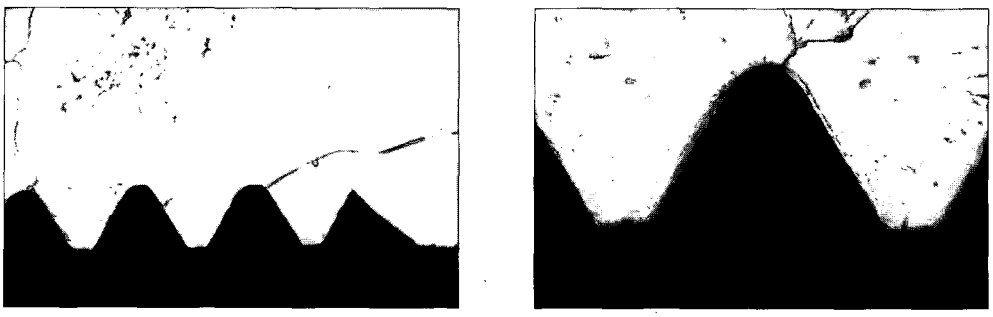
(a) (b)

Fig. 16a and b. (a) Histologic findings of a ground section at the cortical bone site of control group at 4 weeks after implant insertion. Intracortical bone remodeling (toluidine blue stain, original magnification ×100). (b) Thinly down-grown cortical bone (original magnification ×100).



(a) (b)

Fig. 17a and b. (a) Histologic overview of a ground section of PRP group at 8 weeks after implant insertion (toluidine blue stain, original magnification ×40). (b) (original magnification ×100).



(a) (b)

Fig. 18a and b. (a) Histologic overview of a ground section of control group at 8 weeks after implant insertion (toluidine blue stain, original magnification ×40). (b) (original magnification ×100).