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RESEARCH

Combination of platelet-rich plasma with degradable bioactive borate glass for segmental bone defect repair

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Porous scaffold biomaterials may offer a clinical alternative to bone grafts ; however, scaffolds alone are typically insufficient to heal large bone defects. Numerous studies have demonstrated that osteo-inductive growth factor significantly improves bone repair. In this study, a strategy combining degradable bioactive borate glass (BG) scaffolds with platelet-rich plasma (PRP) was tested. The bone defect was filled with BG alone, BG combined with autologous PRP or left empty. Bone formation was analyzed at 4, 8 and 12 weeks using both histology and radiology. The PRP treated group yielded better bone formation than the pure BG scaffold as determined by both histology and microcomputer tomography after 12 weeks. In conclusion, PRP improved bone healing in a diaphyseal rabbit model on BG. The combination of PRP and BG may be an effective approach to repair critical defects.

Keywords : bone defect ; bioactive borate glass (BG) ; scaffold ; platelet-rich plasma (PRP).

INTRODUCTION

Massive bone defects constitute a major challenge to reconstructive surgery. Autogenous bone grafting is considered the gold standard for filling bone defects even today, despite significant problems arising from donor-site morbidity and limited amount of donor bone (*1,5*). Tissue engineering strategies offer a possible alternative to structural bone grafting for the treatment of large segmental bone defects. Scaffolds and growth factors are very important parts of tissue engineering.

More recently, the potential of a borate glass which is produced by replacing the SiO_2 in silicate-based bioactive glass with B_2O_3, has been explored for finding the biomedical applications (*11*). Many studies have showed that the borate glass with controlled and complete degradation behaviour possessed fair biocompatibility and more bio-activity than the silicate-based bioactive glasses and supported the growth and differentiation of human mesenchymal stem cells and were thought to be a new scaffold material for bone tissue engineering (*7,18*).

To improve their osteogenic potential, scaffolds can be combined with growth factors. Growth factors influence the chemotaxis, differentiation,

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proliferation and synthetic activity of bone cells, thereby regulating physiological remodeling and fracture healing. Numerous growth factors, such as bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and insulin-like growth factors (IGF), have a stimulating effect on bone defect healing (*6*). Platelet-rich plasma (PRP) contains a number of these growth factors [PDGF, TGF-β1, TGF-β2, IGF, epidermal growth factor (EGF), and epithelial cell growth factor (ECGF)] in its natural composition (*9*). Because it can be used autogenously, it poses no risk of transmissible diseases. Furthermore, PRP can easily be obtained on the day of surgery by two centrifugation steps from autogenous whole blood. Basic research seems to endorse PRP's ability to support bone and soft tissue healing (*8*).

We hypothesized that platelet-rich plasma (PRP) combined with a degradable bioactive borate glass (BG) would promote functional bone repair. To test this hypothesis, we quantified the effects of implanting the BG in combination with PRP on new bone formation in large segmental defects in a rabbit model, as shown on radiology and histology.

MATERIALS AND METHODS

Animals

Six- to nine-month-old male New Zealand white rabbits (NZWR) were kept in separate cages, fed a standard diet and allowed to move freely during the study. The following groups (each n = 9) were compared : (1) the critical-size defect was filled with a BG scaffold ; (2) it was filled with BG+PRP ; or (3) it was left empty. Animals were treated in compliance with our institutions guiding principles in the care and use of animals. The local ethics committee for animal experiments approved the design of the experiment.

BG scaffolds

The melt-derived borate glass powder (average diameter about 4 μm) with the composition of $6Na_2O–8K_2O–8MgO–22CaO–54B_2O_3–2P_2O_5$ mol% was employed as the scaffolds materials. The details of preparation and the *in vitro* test on this bioactive glass have been reported by Yao *et al* (*19*). The phosphate buffered curding solution (PBCS) with the concentration of 0.38 mol/l $(NH4)_2HPO_4$, 0.09 mol/l $NH_4H_2PO_4$ and the starting pH value of 7.2-7.4 was used. The borate glass powder was then mixed with PBCS. The mixture was placed into 4 mm × 15 mm rubber molds without compression, allowed to harden for 20 min and dry for 24 h forming pellets, and these pellets were ready to be used.

Preparation of platelet-rich plasma (PRP)

Rabbits were anaesthetized by intramuscular injection of 10 mL of ketamine hydrochloride mixed with 5 mL of xylazine at a dose of 0.65 mL/kg body weight. PRP was prepared according to a method previously reported (*10*). Briefly, 10 mL of blood was freshly obtained from 27 rabbits, using a syringe containing 1 mL of acid citrate dextrose-A (ACD-A) solution as an anticoagulant. The blood was centrifuged in a laboratory centrifugation apparatus at 4°C for 10 min at 2400 rpm. Subsequently, the yellow plasma containing the platelet fraction was collected and further centrifuged at 4°C for 10 min at 3600 rpm to separate the platelets. The approximate volume of PRP obtained was 0.8 mL.

Application of PRP

Eighty microliters of freshly thawed PRP was then applied to the BG glasses of the PRP + BG group, and subsequently 20 μl thrombin (0.8 IU activity) calcium chloride (1 M) solution (1:1) was added directly before implantation.

Surgery

The animal model was adapted from Wittbjer *et al* as described previously (*11,12*). Briefly, unilateral 15-mm critical-size defects were created in the distal radial diaphysis. The rabbits were anaesthetized according to the previous described method. An antibiotic (netilmicin 4 mg/kg BW) was administered perioperatively. A superomedial incision of 3 cm was made over the distal radius, soft tissues were dissected, and the bone was exposed by gentle retraction of the muscles. A Hohmann retractor was placed between ulna and radius to protect the ulna. A 15-mm segmental diaphyseal defect was created with an oscillating saw under irrigation with 0.9% sterile saline solution. The periosteum was removed with the bone and 5 mm of periosteum was stripped from each side of the remaining proximal as well as distal main fragment. The defect was irrigated with sterile physiological saline solution, and the ceramic (or cancellous

bone, or nothing) was press fitted into the defect. Muscles, fascia and skin were separately closed over the defect with 3-0 resorbable sutures. Water and food were supplied *ad libitum*. After 4, 8 and 12 weeks, 3 rabbits were killed in each group. After immediate mechanical testing, the specimens were placed in 70% ethanol.

Radiographic evaluation

Standardized anteroposterior and lateral radiographs were taken immediately postoperatively and every 4 weeks thereafter to monitor the placement of the graft and the bony integration.

Micro-computed tomography

Bone formation within the defect regions was also analyzed using micro-CT imaging (GE Micro-CT ; USA). Thresholds were applied to images of each sample to segment newly formed bone from residual scaffold material. A total of 300 slices were acquired for each femur for postmortem scans using a slice increment of 20 mm. The digitized data were analyzed with VG Studio Max 1.2.1 software (Volume Graphics, Heidelberg, Germany), and the amount of new bone formation (newly formed bone voxels per complete tissue voxels of the initially implanted volume) was calculated.

Histological analysis

Bone specimens were placed into 10% neutral phosphate-buffered formaldehyde, decalcified with 10% formic acid, and processed for paraffin embedding. Sections (5 μm thick) were prepared and stained with haematoxylin and eosin to view by light microscopy (AX80T ; Olympus, Tokyo, Japan).

Statistical analysis

Data analysis was performed with SPSS for Windows 11.5 (SPSS Inc., Chicago, IL, USA). Mean values and standard deviations were calculated.

RESULTS

Radiographs at weeks 4, 8, and 12

At week 4, limited bone infiltration from the proximal end towards the center of the defect was observed within constructs from both BG and BG + PRP groups. Visual differences between BG + PRP and BG group were not evident at this early time point. Bone formation within the gap regions increased with time as indicated by radio-

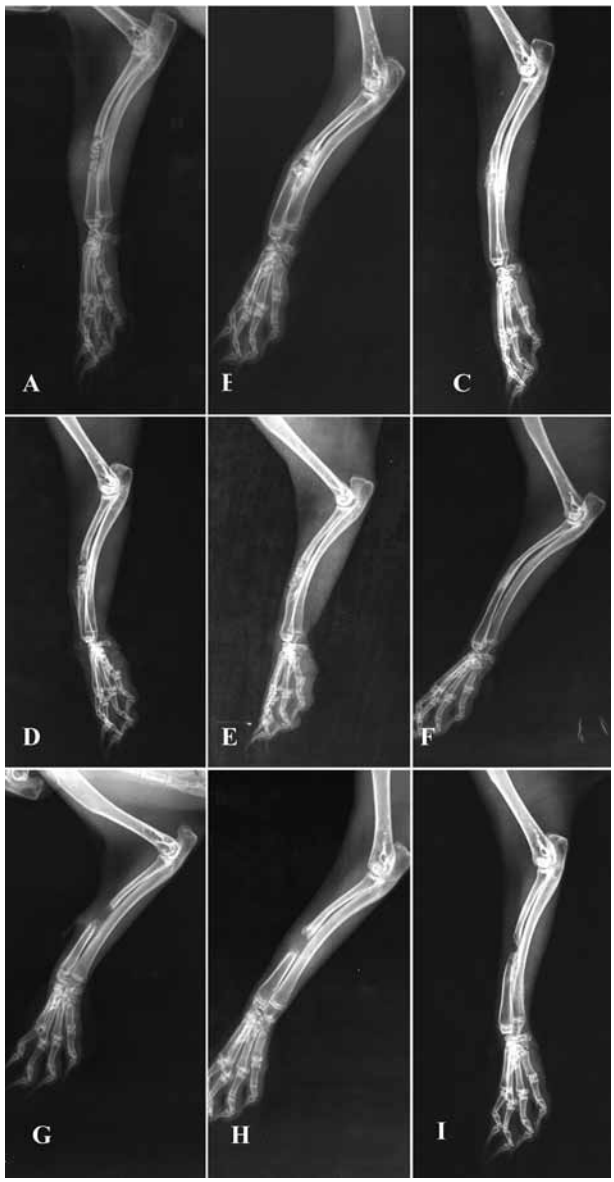

Fig. 1. — Representative radiographs of BG alone (A-C), PRP + BG(D-F) and empty treated (G-I) rabbit radius at 4 (A, D, G), 8 (B, E, H), and 12 (C, F, I) weeks after surgery. Initially, limited bone infiltration from the proximal end toward the center of the defect was observed within constructs from both groups. Bone unions in PRP+BG treated radius were noted at 12 weeks after surgery.

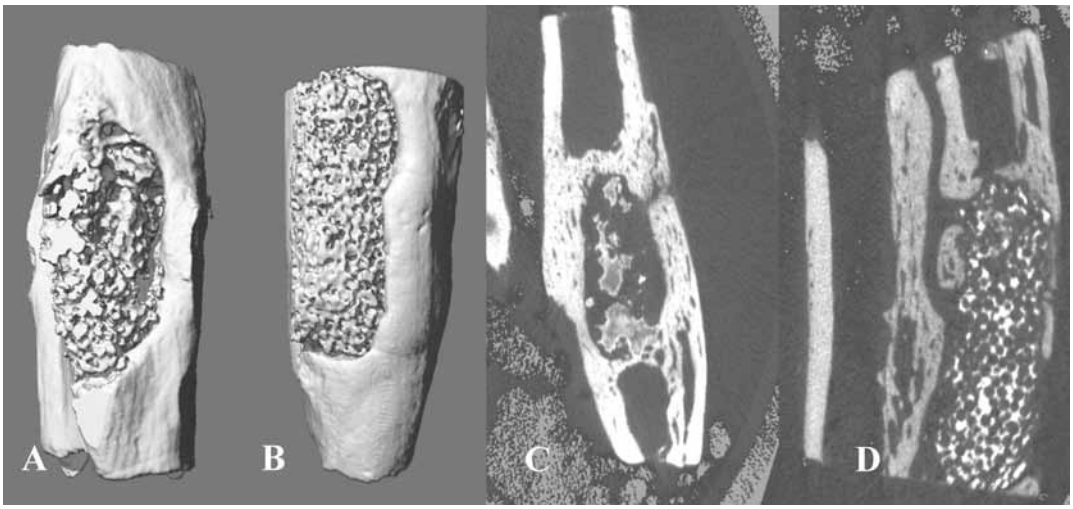

Fig. 2. — Representative week 8 postsurgery micro-CT images of PRP + BG (A, C) and BG (B, D) group. Note the bone union and callus formation in PRP + BG were better than that in the BG group.

graphs taken at 8 and 12 weeks. At 12 weeks, complete bone bridging was qualitatively evaluated in a blinded fashion by two observers, based on the absence of a gap in radiodensity. Eight out of the nine PRP-treated radius exhibited apparent bony union. In contrast, bone bridging was observed in only four out of the nine control radii in the BG group. In the empty group, the new bone formation was minimal (Fig. 1).

Micro-CT analysis of new bone formation at week 12

At 12 weeks, the volume of the newly formed bone in the BG + PRP animals was significantly higher than in those receiving only BG ($p < 0.05$) (Fig. 2).

Histological results

In general, the histological findings were in accordance with the results of m-CT analysis and the radiographs (Fig. 3). There was little or no new bone formed in the empty defects. The BG + PRP composite transplant integrated with the local bone, proximally and distally. The area of newly formed bone was higher in defects treated with BG + PRP than in defects treated with BG alone ($p < 0.05$). The histology specimens were examined for the presence of lymphocytes or multinucleated giant cells/macropaghes. There were no differences between the groups. The bone tissue formed in the periphery of the glass pores without a fibrous layer between the bone and the glass. This indicates that the BG scaffold has osteoconductive properties.

DISCUSSION

It has been a great challenge to repair large bone defects using artificial biomaterials that do not induce donor site morbidity and are easy to handle. In a previous study, we have studied a novel artificial scaffold, a degradable bioactive borate glass. This BG showed potential as an artificial bone scaffold for repair of large bone defects, although complete restoration of bone union was not achieved. The purpose of the present study was to examine the efficacy of a combination of BG and PRP for the repair of large bone defects in the rabbit model. The present findings indicate that the combination of PRP and BG had a significantly positive effect on bone formation as shown on histology and micro-CT.

Various biomaterials have been used as scaffolds for the repair of bone defects, including tricalciumphosphate (TCP), hydroxyapatite (HA), polylactic

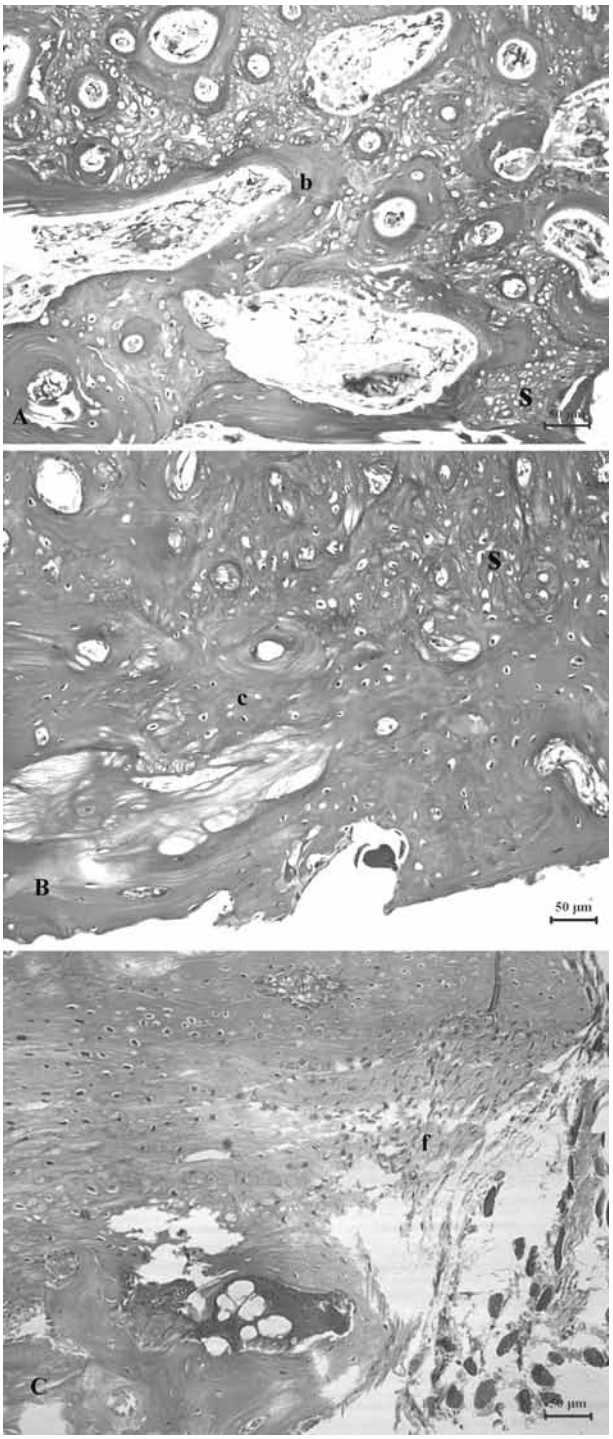

Fig. 3. — Histological sections of radius defects 8 weeks after application of PRP+BG (A) and BG (B) or left empty C). b, bone ; s, unresorbed borate glass scaffold ; c, cartilage ; f, fibrous tissue.

acid (PLA), polycaprolacton (PCL), etc. Most candidate artificial biomaterials have been too rigid or fragile, resulting in long-term problems due to poor matching of biomechanical properties between the implant and the adjacent bone tissue. Moreover, it is difficult to devise bioabsorbable materials with high mechanical strength, because they are designed to degrade and disappear over a period of several months. Therefore, the mechanical properties of biomaterials are very important in cases with cartilage defects under conditions of load bearing. The BG used in the present study shows promise as a biomaterial, because it has high break strength despite its low modulus of elasticity and downward convex J-shaped stress-strain curve, indicating that it behaves more like natural bone than like conventional artificial biomaterials. In a recent study of the use of BG as an artificial biomaterial in a rabbit model, it was found to have biomechanical and morphologic biostability, appropriate viscoelasticity, and excellent fatigue properties (*3,4,5,14*). These findings suggest that the properties of BG biomaterial may be suitable for clinical application in large bone defects. However, the BG must also reconstruct the bone vascularization and structure, because growth factors and cells also play an important part in bone formation. One possible way to achieve this dual function is to combine a biomaterial that has appropriate mechanical properties (for mechanical support) with a growth factor that induces bone formation.

PRP serves as a potential source of these factors and upon contact with thrombin and calcium ions, results in a large release of prepackaged growth factors into the immediate local environment. Platelets contain angiogenic, mitogenic, and osteogenic growth factors in their granules (*2,12,14*) and function as a reservoir of natural growth factors, which can be secreted when activated by substance contact and stimulus actions (*18*). Among the growth factors of platelets, TGF-β has an inherent activity to inhibit bone resorption, osteoclast formation, and osteoclast activity as well as to trigger rapid maturation of collagen at an early stage of wound repair (*3,15*). PDGF increases the population of wound-healing cells and recruits other angiogenic growth factors to the wound site (*15*). Taken together, platelets are a

promising source of autologous growth factors. Platelets are readily concentrated as the PRP from blood. Due to its autologous origin PRP is an attractive alternative to other osteoinductive agents without the risk of allergies and graft versus host reactions. Besides, the use of PRP does not result in essential additional costs. Previous studies and case reports indicate that PRP has favorable effects on promotion of early craniofacial bone healing (*15*), the most convincing being the human studies by Marx (*13*). In the present study, the results confirmed the favorable effects on bone formation. We found the addition of PRR could further increase the rate of bone formation in our model.

In conclusion, this study showed that PRP combined with BG has lead to a significantly better bone regeneration compared to isolated application of BG in an *in vivo* critical size defect on long bones of rabbits during our chosen time frame of 12 weeks. Furthermore the preparation and application of PRP represent an easy and successful method to promote bone healing in this model.

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