Bone Regeneration in Peri-Implant Defect Using Autogenous Tooth Biomaterial Enriched with Platelet-Rich Fibrin in Animal Model

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Featured Application: Autogenous tooth biomaterial can be used as graft material in dental implant surgery.

Abstract: Tooth biomaterial may be useful in bone regeneration for restoring peri-implant defects in vivo. The aim of this study was to compare bone regeneration capacity in peri-implant defects augmented with autogenous tooth biomaterial combined with platelet-rich fibrin (PRF), tooth biomaterial alone, or PRF alone. Two monocortical defects were generated on each tibia of 10 New Zealand white rabbits (n = 10 per group) with a trephine bur, and the dental implant was installed into the defects. In experimental groups 1, 2, and 3, the peri-implant defects were filled with tooth biomaterial and platelet-rich fibrin (PRF), tooth biomaterial only, and PRF only, respectively and the control was left empty. Micro computed tomography (CT), implant stability, and histomorphometric analysis were conducted eight weeks after operation. The mean regenerated bone areas were 53.87 ± 7.60%, 51.56 ± 6.45%, and 18.45 ± 1.34% in experimental groups 1, 2, and 3, respectively, and 11.57 ± 1.12% in the control. Mean bone-to-implant contact values were 43.67 ± 2.50%, 41.07 ± 2.59%, and 21.45 ± 1.25% in experimental groups 1, 2, and 3, respectively, and 16.57 ± 2.83% in the control. Tooth biomaterial enriched with platelet-rich fibrin (PRF) and tooth biomaterial alone showed more enhanced regeneration than PRF alone in our study.

Keywords: tooth biomaterial; platelet-rich fibrin; bone regeneration; graft material

1. Introduction

Bone graft materials are essential for treatment in oral and maxillofacial surgery because of the implant unavailability for bone replacement [1]. The development of bone graft materials has increased the available bone regeneration technologies in the dental field. The use of bone grafts during immediate post-extractive implant placement has shown better aesthetic results [2]. Autogenous bone graft is regarded as standard for bone regeneration in considerable peri-implant bone defects [3]. However, this approach is limited when the volume of the defect exceeds the amount of attainable graft and donor site morbidity [4]. Therefore, alternatives to autogenous bone grafts are needed [5]. Various bone graft materials that promote adequate bone healing have been reported in the field of oral and maxillofacial surgery [6]. Allogeneic, xenogeneic, and synthetic bone have been developed and used as bone substitutes in oral and maxillofacial surgery [7,8]. Experiments to identify the best
bone graft material are underway. Furthermore, a bone graft material derived from autogenous tooth has been developed [8].

A tooth-derived bone graft or tooth biomaterial is a type of autogenous bone graft fabricated from an extracted tooth with a demineralization process. Tooth biomaterial may be useful as a graft material for bone regeneration because of its osteoconductive properties [9]. A previous study showed that the primary tooth pattern is formed by hydroxyapatite (HA) as well as small amounts of $\beta$-tricalcium phosphate, octacalcium phosphate, and amorphous calcium phosphate; although the level of HA crystallization varies according to the area of the tooth [9]. HA can be degraded and absorbed in vivo. Bone and teeth have a very similar chemical composition. The inorganic and organic content of dentin and alveolar bone are alike so it can easily replace autogenous bone grafts. The only difference is the method of harvesting the graft. While harvesting a bone graft from other sites can cause recipient site morbidity, tooth bone graft can be easily prepared from a non-restorable or third molar extracted tooth. Tooth biomaterial is biocompatible and has been used as a bone grafting material to regenerate bony defects [10].

Autogenous bone grafts have been used in combination with bioactive glass [11] and bovine bone grafts [12] to enhance the bone volume fraction. When autogenous bone graft is used in combination it offers certain advantages like increased volume of the graft and obviates the need for large amounts of autogenous bone [13]. Bone grafting techniques using platelet-rich plasma (PRP) have been shown to improve bone healing in maxillofacial surgery [14,15]. Some studies successfully applied PRP in bone grafting surgery [16,17]. Various growth factors released from PRP, like platelet-derived growth factor, insulin-like growth factor, and epidermal growth factor have bone regeneration effects on peri-implant bone grafts [18,19]. However, some studies showed that PRP did not have bone regeneration effects on peri-implant bony defects [20]. Platelet-rich fibrin (PRF) is a second-generation platelet concentrate that can be isolated by centrifugation from autogenous blood. PRF is an autogenous fibrin clot rich in platelets and does not require factors such as bovine thrombin [21]. The use of PRP gels (fibrin) in bone defects does not enhance healing of the bone defect compared to non-treated defects [22]. However, the application of additional fibrin with biomaterial can increase bone regeneration compared to biomaterial alone. Significant fibrin sealant-mediated enhancement of bone repair was observed in a previous study [23]. Although platelet cytokines play an important role in enhancing bone regeneration, the fibrin matrix that supports these molecules determines the element responsible for the therapeutic potential of PRF [24,25]. Tooth biomaterial is a useful source of bone graft material for increasing bone regeneration effects. In the present study, a powder type of tooth biomaterial was used with PRF to restore peri-implant bony defects. The purpose of this study was to evaluate bone regeneration with a tooth biomaterial and PRF in peri-implant bone defects.

2. Materials

The Institutional Animal Care and Use Committee of Korea University approved this study protocol (KUIACUC-2010-162). Animal selection, management, and the surgery protocol were approved by the Institutional Animal Care and Use Committee of Korea University, Seoul, Korea.

Ten 4-month-old New Zealand white rabbits *Oryctolagus cuniculus* weighing approximately 3 kg were used for this study. Animals were kept individually in their cages designated with a number at the animal research lab. They were closely monitored by a veterinarian in the lab and received adequate water and diet under 12-h light/dark cycle.

A powder type of tooth biomaterial was prepared by the Korea Tooth Bank Co. (Seoul, Korea). After extraction, the teeth were sent for processing submerged in ethyl alcohol. Any attachments on the tooth surface like soft tissue or foreign material were removed. The tooth was divided into two parts the crown and the root and crushed to form a powdered bone graft. Then it was required to undergo dehydration, defatting, and lyophilization, and was finally packed in a capsule after sterilization.
Two monocortical defects were created on each tibia. The defects were randomly assigned into four groups: tooth biomaterial combined with platelet-rich fibrin (PRF) ($n = 10$), tooth biomaterial alone ($n = 10$), PRF alone ($n = 10$), and empty control ($n = 10$).

2.1. Methods

Intramuscular injection with a combination of 0.4 mL of ketamine and 0.3 mL of xylazine was used as general anesthesia. For preparation of PRF, 10 mL blood sample from the ear vein of each rabbit were procured and centrifuged for 12 min at $400 \times g$. After centrifugation of blood, three distinct layers were formed. The PRF was collected from the middle layer. The surgical area was first shaved, and then povidone-iodine antiseptic was used for disinfection. An incision was made in the periosteum. The tibia bone was exposed by sharp subperiosteal dissection. Two bone holes in the tibia were formed monocortically with a trephine bur. A distance of 5 mm was kept between the two holes. About 2 mm of peri-implant defect were created on each side. A dental implant, 8 mm in length and 3 mm in diameter (Dentium®, Seoul, Korea), was installed in these defects (Figure 1). The groups were randomly assigned. All implants were anchored to the opposing cortex. The peri-implant gap in experimental group 1 was filled with tooth powder and PRF. In experimental group 2 tooth powder alone was used in the defect. The peri-implant defect around the implant in experimental group 3 was filled with PRF alone, while that of the control group was left empty. The implantation site was uniformly allocated and randomized. A 3-0 silk suture was used for closing periosteum and skin. Postoperatively, gentamicin 1 mg/kg was delivered intramuscularly 3 times a day for 3 days. The animals were sacrificed after 8 weeks. The specimens were removed using a round-shaped bur, and then examined grossly for inflammation. After microscopic computed tomography ($\mu$-CT) examination, the specimens were fixed in 10% buffered formalin solution for histomorphometric evaluation.

2.2. Histomorphometric Evaluation

Specimens were prepared as described previously. After staining and dehydration, specimens were embedded with methyl methacrylate resin. To prepare the implant specimen, a low speed diamond wheel saw was used and the specimen was sliced along its long axis and further grounded to a thickness of 30 µm. Images were captured using a digital camera (DP-20; Olympus, Tokyo, Japan) and evaluated with Sigma Scan Pro software (SPSS, Inc., Chicago, IL, USA). The amount of new bone formed was determined based on the percentage of the total area between the consecutive threads. Evaluation images from three abutments portion were used. The bone-to-implant contact was defined as the total percent of bony contact to the implant thread.

2.3. Stability Evaluation

The Periotest system was used for studying the osseointegrative stability of implant fixtures. The top of the implant served as a standard abutment in the Periotest measurements. The Periotest hand piece (Periotest, Siemens AG, Bensheim, Germany) sleeve was set at a pre-defined distance for measurement centered perpendicularly to the long axis of the implant. For better control of the handpiece, the stand position was affixed. Five measurements were registered in a chart for each sample following the operating manual to attain an average value.

2.4. Statistical Analysis
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2.4. Statistical Analysis

The histomorphometric and stability evaluation results were depicted as mean ± standard deviation. The quantitative data were compared among the groups using analysis of variance by ranks followed by Tukey’s post hoc test. In addition, $p$-values $<0.05$ were considered statistically significant. We used IBM SPSS Statistics for Windows, (version 22.0, IBM Corp, Armonk, NY, USA) for analysis.

3. Results

3.1. Clinical and Micro-CT Observations

All 10 rabbits remained healthy throughout the process and the surgical sites healed without any adverse effects or visual signs of inflammation. The specimens were retrieved after a period of eight weeks. Images were acquired with Skyscan™ showing lateral views of the defects. Regenerated new bone was observed in periphery and proximity to the adjacent host bone in the experimental groups. The relative defect size was small in group 1 and group 2 (Figure 2).
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Figure 2. Micro computed tomography (CT) images of regenerated new bone in peri-implant defects at 8 weeks. (A) Defect filled with tooth biomaterial and platelet-rich fibrin (PRF). (B) Tooth biomaterial was grafted into defect. (C) The PRF was grafted into the defect. (D) Unfilled defects around the implant neck served as controls.

3.2. Histologic Result
In groups 1 and 2 bone formation was observed. In group 1 augmented areas were constructed by newly formed bone bridges, covered with new bone. Group 2 showed a considerable amount of cortical bone regeneration. The bone defect was approximately filled by residual tooth bone graft material. In group 3, in the crestal part of alveolus, the graft separated by a compact bone covering and a soft tissue gap can be spotted. In group 4 implant-to-bone contact was lower (Figure 3).
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Figure 3. Histologic view at 8 weeks (original magnification 12.5×): (A) group 1; (B) group 2; (C) group 3; and (D) group 4.

3.3. Histomorphometry

The area of regenerated new bone and implant-to-bone contact was less in the control group than the experimental groups (Table 1). However, cortical bone regeneration was high in the other experimental groups. Table 1 shows the histomorphometry results. The mean values of new bone formation were 53.87 ± 7.60%, 51.56 ± 6.45%, and 18.45 ± 1.34% in experimental groups 1, 2, and 3, respectively, and 11.57 ± 1.12% in the control. The difference between group 1 and the control was significant ($p = 0.003$).
### Table 1. Histomorphometric analysis.

| Group                        | Regenerated Bone Area (%) | Bone to Implant Contact (%) |
|------------------------------|---------------------------|-----------------------------|
| 1. Tooth Biomaterial + PRF   | 53.87 ± 7.60              | 43.67 ± 2.50                |
| 2. Tooth Biomaterial         | 51.56 ± 6.45              | 41.07 ± 2.59                |
| 3. PRF                       | 18.45 ± 1.34              | 21.45 ± 1.25                |
| 4. Control                   | 11.57 ± 1.12              | 16.57 ± 2.83                |

Data are presented as the mean ± standard deviation.

### 3.4. Stability Evaluation

The implant stability test results are shown in Table 2. Periotest values equal to or less than 0 (−8 to 0) signify good osseointegration while values in the range of 1 to 9 are an indication to avoid application of pressure on the implant. The average Periotest values were higher in group 1 than the other groups after eight weeks of operation. The Periotest values of experimental groups 1, 2, and 3 and the control were −3.40 ± 0.31, 1.25 ± 0.07, 4.79 ± 0.93, and 5.97 ± 0.81, respectively. The difference between group 1 (p = 0.006) as compared to the control was statistically significant.

### Table 2. Measurement of implant stability.

| Group                        | Periotest Values |
|------------------------------|------------------|
| 1. Tooth Biomaterial + PRF   | −3.40 ± 0.31     |
| 2. Tooth Biomaterial         | 1.25 ± 0.07      |
| 3. PRF                       | 4.79 ± 0.93      |
| 4. Control                   | 5.97 ± 0.81      |

Data are presented as the mean ± standard deviation.

### 4. Discussion

To prevent alveolar bone loss and reduce the total time period of treatment, immediate implant installation after tooth extraction has been suggested [26,27]. After tooth extraction following periodontal disease, alveolar bone gradually decreases in height and width. Alveolar bony defects cause problems for immediate implant installation. Tooth biomaterial composition is similar to that of bone consisting of hydroxyapatite and calcium phosphate minerals. Various growth factors like bone morphogenetic protein, insulin-like growth factor, platelet-derived growth factor, fibroblast growth factor, and transforming growth factor are found in the tooth [28]. In this study, tooth biomaterial and PRF were used in the self-created peri-implant bony defects. The tooth biomaterial groups (groups 1 and 2) showed higher new bone formation, bone-to-implant contact area (Table 1), and lower Periotest values (Table 2). The histomorphometric results indicated that bone formation in groups 1 and 2 was greater than the unfilled control. Only a single cut from a histologic section can be observed for osseointegration rather than full geometric information. Measurement of implant stability further helps in evaluating osseointegration. Implant stability in groups 1 and 2 was significantly higher as compared to the control group. Dense cortical bone offers greater support for dental implants in comparison to cancellous bone. In addition, it exhibits increased removal torque [29]. An implant surrounded with cancellous bone exhibits lower holding strength than an implant which is connected by a few threads [30]. Due to lack of exercise in caged lab animals, the middle of the tibia lacks thick cortical bone [31]. A bicortically installed implant exhibits significantly greater removal torque as compared to monocortically installed implant [32]. The experimental group showed a higher stability value, possibly because of coronal cortical bone regeneration. Bone grafting biomaterials and cellular therapy have been studied to regenerate bone in peri-implant defects. Bone grafting biomaterials such as bioactive glass have been used to restore peri-implant defects and showed beneficial results [33].
No effect was observed in peri-implant bone healing when a prepared socket was embedded with cells seeded with growth factor [34]. This may be due to the higher solubility of growth factor, and difficulty in achieving therapeutic concentration for a longer time period. PRP showed significant results for restoring peri-implant defects when applied in combination with different bone grafts [35].

In bone graft techniques, PRF may be a good reservoir as a biomaterial. PRF is not related to immune reactions because it can be obtained from autogenous donors. Platelets release various cytokines like platelet-derived and insulin-like growth factors [36,37]. PRP promotes osteoblast proliferation and may be clinically applicable for bone graft procedures in implant dentistry [38]. PRF can induce bone healing and may induce bone regeneration. Lee et al. successfully repaired peri-implant defects by application of PRF alone [39]. However, there was no considerable difference between experimental group 3, where only PRF was applied, and the unfilled control group in this study. Similar results were observed in another study, where platelet-rich fibrin did not increase bone regeneration in non-critical size defects in rabbit tibia. The authors suggested that this may be partially due to difficulty in retention of PRF at the defect site because of the anatomy of the tibia [40]. The differences in the biology and physiology can also affect the outcomes. Furthermore, no statistically significant difference was observed between groups 1 and 2. The experimental difference between the two groups was the application of PRF. There is a need to further investigate the effects of PRF when used in bone defects.

To increase the release of growth factors, an appropriate scaffold is required. The tooth biomaterial led to the successful formation of new bone [10]. The powder form of a tooth biomaterial can be used for a narrow bony defect with or without PRF. Bioactive glass or HA biomaterials do not biodegrade fully and tooth biomaterials also require a comparatively longer duration for complete biodegradation [35].

In this study, a tooth biomaterial was successfully applied for restoration of peri-implant defects. We proved that peri-implant defects were successfully restored by using the tooth biomaterial enriched with PRF. Tooth biomaterial alone also exhibited satisfactory results as compared to PRF alone and empty defects. These results were supported by the implant stability test. More extensive clinical studies are needed to assess the application of tooth derived biomaterial as a bone graft material for bone tissue engineering. Further studies applying PRF in tissue engineering techniques should be carried out to verify this treatment method.

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