Platelet-rich fibrin combined with a particulate bone substitute versus guided bone regeneration in the damaged extraction socket: an in vivo study

Kyu-Jin Hong

Department of Dentistry

The Graduate School, Yonsei University

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Directed by Professor Jung-Seok Lee

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Thesis Supervisor: Jung-Seok Lee

Seong-Ho Choi

Ui-Won Jung

Jae-Kook Cha

Jeong-Won Paik

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Yonsei University
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감사의 글

본 연구가 학위 논문으로 출판이 되기까지 어려움이 있을 때마다 아낌없는 조언과 가르침을 주시고 늘 정신적 멘토가 되어주신 이중석 교수님께 진심으로 감사 드립니다. 또한, 제가 치주과 수련을 무사히 마칠 수 있도록 지도해 주신 조규성, 최성호, 김창성, 정의원, 차재국, 백정원, 송영우, 박진영 교수님께도 감사의 말씀을 전합니다.

실험, 임상 등 여러 방면에서 도움을 주신 연구원 선생님들과 오늘 이 글을 쓰기까지 수많은 시간을 함께 해온 의국원들께 감사 드립니다.

마지막으로 제 모든 날, 모든 순간에 항상 옆에서 넘치는 사랑과 응원으로 격려해 준 남편, 그리고 언제나 든든한 울타리가 되어주는 가족에게 감사와 사랑의 마음을 전합니다.

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저자 홍규진

TABLE OF CONTENTS

List of figures

List of table

Abstract

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Kyu-Jin Hong, M.S.D.

Department of Dentistry
The Graduate School, Yonsei University

(Directed by Professor Jung-Seok Lee, D.D.S., M.S.D., PhD.)

Aim: It has been proposed that platelet-rich fibrin (PRF) can be used to support bone regeneration during alveolar ridge augmentation. The aim of this study was to determine whether an approach utilizing PRF provides similar performance to the established guided bone regeneration (GBR) procedure.

Materials and Methods: Two-wall defects were surgically created in beagle dogs and treated in three experimental groups: (i) a sticky bone (SB) substitute prepared using liquid PRF and deproteinized porcine bone mineral (DPBM); (ii) SB covered with solid PRF compressed by a collagen membrane. Quantitative reverse-transcription polymerase chain

reaction was applied to the specimen after 1 week of healing, and microcomputed tomography (micro-CT) and histological outcomes were analyzed after 8 weeks of healing. Repeated-measures analysis of variance (RM-ANOVA) and Friedman test were used to compare the experimental groups. Results were considered statistically significant when $p < .05$ in a two-tailed test.

Results: Compared with GBR, PRF resulted in a moderate increase in the expression levels of osteoblast and osteoclast markers, osteocalcin and calcitonin receptor. Moreover, PRF modestly increased angiogenesis and the inflammation markers vascular endothelial growth factor (VEGF) and IL-6. Micro-CT and histological analyses confirmed the expected increased alveolar ridge area (109.96±20.13%, 107.47±18.70%, and 104.57±17.49% in micro-CT analyses, and 120.01±25.31%, 117.59±21.70%, and 114.56±25.48% in histologic analyses of the baseline in the GBR, SB, and SB+PRFM groups, respectively) with no significant differences between the three groups. Consistently, graft consolidation, as indicated by new bone formation at the defect site, did not differ significantly between groups.

Conclusions: The present results demonstrate that PRF-based approaches perform comparably to the established GBR procedure in terms of the consolidation of DPBM in two-wall alveolar defects.

Keywords: animal model, dental implants, histology, molecular biology, therapy

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Kyu-Jin Hong, M.S.D.

Department of Dentistry
The Graduate School, Yonsei University

(Directed by Professor Jung-Seok Lee, D.D.S., M.S.D., PhD.)

I. INTRODUCTION

Platelet-rich fibrin (PRF) is increasingly being used in the clinical dental field in various regenerative procedures, including alveolar ridge regeneration, implant placement, and sinus floor elevation (Castro et al., 2017; Dragonas et al., 2019; Strauss et al., 2018). Although these procedures can be performed successfully by utilizing the currently available biomaterials, it is desirable to further enhance wound healing and accelerate bone regeneration, especially in challenging bone defects. The rationale for using PRF is that it consists of a dense fibrin network encompassing platelet and leukocyte concentrates that

release above-physiological levels of bioactive factors that are essential for wound healing and tissue repair (Pavlovic et al., 2021).

For alveolar ridge preservation, there is clinical evidence that the grafting of multiple layers of PRF into the extraction socket can enhance radiographic bone fill, reduce dimensional shrinkage of the alveolar ridge (Alzahrani et al., 2017; Temmerman et al., 2016), and minimize post-operative pain (Marenzi et al., 2015; Temmerman et al., 2016). However, a recent clinical trial found that applying PRF alone in the extraction socket did not prevent the reduction of alveolar ridge dimensions after tooth extraction, resulting in no difference relative to no grafting (Castro et al., 2021). This unsatisfactory outcome can be attributed to the early degradation of PRF and poor space maintenance. In the extraction socket, rapidly resorbing biomaterials have been suggested to be less effective for preserving alveolar ridge dimensions (Araujo & Lindhe, 2011).

Two PRF preparations have been introduced for clinical applications: (i) compression of the fibrin clot into a membrane (Hatakeyama et al., 2014), and (ii) using a liquid coagulant as a binder to form a sticky mixture with particulate bone substitutes (Wang et al., 2021). This sticky bone (SB) substitute can be beneficial for bone augmentation procedures since the putty-like mixture provides additional stability, can be molded into the specific shape of each defect, and is easy to handle. The bone graft particles in SB would help to strengthen the PRF, especially when placed in unfavorable defect configurations such as the damaged extraction socket (Lee et al., 2018; Lee et al., 2015; Tien et al., 2021). Moreover, SB has been shown to produce superior new bone formation and dimensional stability relative to using a PRF membrane (PRFM) (Oliveira et al., 2015).

The pre-clinical extraction socket model has been well documented in several studies with various defect configurations and biomaterials (Araujo & Lindhe, 2005; Araujo et al., 2005; Lee et al., 2018; Tien et al., 2021). A series of histological studies found that the dimensional shrinkage of an extraction socket can be reduced by grafting osteoconductive bone substitutes. Moreover, using a collagen membrane to cover the socket entrance

improved the quality of regenerated bone as required in guided bone regeneration (GBR) (Avila-Ortiz et al., 2019; Thoma et al., 2019). In light of the aforementioned benefits of PRF, we hypothesized that adding PRF to a scaffolding biomaterial will enhance the dimensional stability and the quality of new bone formed in the two-wall damaged extraction socket more than when using GBR. To test this hypothesis, the areas of new bone formation and the total augmented tissue were assessed as the primary and secondary outcomes, respectively.

The objective of this study was to determine the effects of SB prepared using PRF and deproteinized porcine bone mineral (DPBM) in combination with a PRFM on the regeneration of two-wall damaged extraction sockets.

II. MATERIALS AND METHODS

1. Animals and materials

Seven beagle dogs (weighing about 15 kg, aged 15–24 months) were included in this study. They were individually housed at a standardized temperature and humidity and were provided with a standardized soft diet. Six of the animals were used for radiographic and histological analyses, and one was used to evaluate gene expression during the initial healing phase. The sample size was determined based on a previous study (Tien et al., 2021) in adherence with the Three R's principle in animal research: replacement, reduction, and refinement. The study design was based on the ARRIVE guidelines (Percie du Sert et al., 2020) and approved by the Institutional Animal Care and Use Committee of Yonsei Medical Center in Seoul, South Korea (Approval No. 2018-0329).

A particulate bone substitute (DPBM; THE Graft, Purgo Biologics, Seongnam, South Korea) and a non-cross-linked porcine collagen membrane (BioCover, Purgo Biologics) were used for GBR. Whole blood was drawn from the jugular vein of the animal using two sterile glass vacuum tubes (10 mL/tube) and was centrifuged at 1,300 rpm for 8 min (DUO Quattro, PRF Process, Nice, France) to obtain the PRF. Two types of commercially available tubes for the production of advanced-PRF (A-PRF) and injectable-PRF (I-PRF) (PRF Process) were used to obtain one of the two types of PRF: a fibrin mass and a liquid type, respectively. The A-PRF tube has a glass inner surface that activates the coagulation cascade during centrifugation, whereas the I-PRF tube has a plastic surface that does not activate the coagulation cascade, resulting in a yellow liquid containing high concentrations of platelets and leukocytes (Fujioka-Kobayashi, Miron et al. 2017). The fibrin mass was collected from the A-PRF tube and gently compressed using a metallic tray (Steribox & Instruments, PRF process, AR Deutsehland, Germany) to obtain the PRFM. The I-PRF was extracted from the top layer of the I-PRF tube and combined with the

exudates that oozed from the fibrin mass during the compression process, which was then added to DPBM in a metal tray. A moldable biomaterial mass (i.e., SB) was produced by mixing DPBM with these liquids at a volume ratio of 1:1 using a metal spatula for 2-3 min until the mixture acquired a firm and sticky consistency (Fig.1). SB was applied to the bone defect immediately after being prepared.

2. Study design

Two-wall damaged extraction socket models were used in this study in accordance with a previously reported protocol (Tien et al., 2021). Briefly, the distal roots of the unilateral mandibular second, third, and fourth premolars were extracted after performing tooth hemisectioning, and the entire buccal and lingual bone walls were removed (Fig. 1). The mesial roots were preserved to represent the pristine site and to provide a baseline for dimensional change measurements in the corresponding distal grafted sites. Three damaged extraction sockets were randomly allocated to the following groups:

i. GBR group: socket grafted with DPBM and covered with a collagen membrane (positive control);
ii. SB group: socket filled with the sticky DPBM and the liquid PRF mixture;
iii. SB + PRFM group: socket filled with SB and then covered with a PRFM.

Randomization was performed for the group allocation in the first dog, and then for the groups were rotationally allocated in the remaining dogs so that they were evenly distributed to the three premolar sites.

3. Surgical protocols

General anesthesia was induced using medetomidine (0.75 mg/kg, IM; Tomidin, Provet Veterinary Products, Istanbul, Turkey) and alfaxalone (2 mg/kg, IV; Jurox,

Rutherford, NSW, Australia), and maintained using isoflurane (Forane, Choongwae Pharmaceutical, Seoul, South Korea) inhalation. After inducing local anesthesia using 2% lidocaine hydrochloride at a dilution of 1:80,000 (Kwangmyung Pharm, Seoul, South Korea), a full-thickness flap was elevated only at the experimental site of the distal root region. The distal roots of the second, third, and fourth mandibular premolars were extracted after hemisectioning, and the mesial roots were decoronated and maintained. Two-wall socket defects were surgically constructed by completely removing both buccal and lingual bone plates using a diamond bur (Fig. 1). After the defect preparation, biomaterials were applied according to the experimental group allocation: GBR, SB, or SB+PRFM. The flap was repositioned, and primary closure was achieved using 6-0 monofilament sutures (Monosyn, B. Braun Medical, Bethlehem, PA, USA). Antibiotics (20 mg/kg; cefazolin sodium, Yuhan, Seoul, South Korea) and analgesia (0.2 mg/kg; meloxicam, Boehringer Ingelheim, Ingelheim, Germany) were administered once daily for 7 days after surgery, and the sutures were then removed. The animals were observed by veterinary professionals throughout the healing period, with the wounds examined under intravenous sedation on a weekly basis for detecting any abnormalities, at which time oral prophylaxis was applied. The animals were then killed, one after 1 week of healing for gene expression analysis, and the other six after 8 weeks for radiographic and histological analyses.

4. Microcomputed tomography radiographic analysis

The collected samples were fixed in a 10% neutral buffered formalin solution for 2 weeks. Microcomputed tomography (micro-CT) scanning (SkyScan 1173, SkyScan, Aartselaar, Belgium) was performed (field of view = 6.2 cm; projection time = 40 min and 41 seconds; number of projections = 799; frame averaging = 4) at a resolution of 35 μm (achieved using conditions of 130 kV and 60 μA). The data were converted into the DICOM format and uploaded to a computer software for three-dimensional

reconstruction (OnDemand3D, Cybermed, Seoul, South Korea). The ridge dimensions surrounding the mesial root were used as the pre-extraction baseline for post-operative dimensional change measurements at the distal grafted site based on the assumption that there were negligible differences in the original ridge dimensions at the mesial and distal sites of each tooth. The cross-sectional images obtained from the mesial root (baseline) and centre of the grafted site (after 8 weeks) were superimposed using the mandibular canal and the mandible outline as references (Fig. 2A). The area of interest was demarcated coronally and apically at the ridge crest and dental apex, respectively. Proportional changes in the ridge area from baseline to 8 weeks were calculated using computer software (Adobe Photoshop CC 2020, Adobe Systems, San Jose, USA).

5. Histological analysis

Each unilateral mandible was sectioned into six blocks containing three mesial pristine sites and three grafted sites after decalcification. The samples were trimmed and embedded in paraffin after dehydration using a series of sequentially graded ethanol solutions. Histological slides were prepared from the most central region of the blocks and were stained with hematoxylin, eosin, and Masson's trichrome. The slides were digitally scanned at ×200 magnification (PANNORAMIC 250 Flash III, Budapest, Hungary). The digitally scanned images were analysed histomorphometrically using computer software (Adobe Photoshop CS5, Adobe Systems). Histological images from the mesial pristine and distal experimental sites were superimposed using the mandibular canal and the mandible outline as references (Fig. 2B), and the following planimetric dimensions were measured:

- Augmented ridge area (ARA): demarcated by the outermost margin of the grafted site;

- Regenerated ridge area (RRA): demarcated by the outermost margin of the newly formed bone;

- Residual biomaterials: the area of the residual DPBM particles relative to the ARA;

- Mineralized tissue: the area of newly formed bone relative to the RRA;

- Fibrovascular tissue: the area of non-mineralized tissue relative to the RRA.

6. Quantitative reverse-transcription polymerase chain reaction

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed to examine the mRNA expression levels of target genes encoding bone morphogenic protein-2 (BMP-2), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), fibroblast growth factor-2 (FGF-2), osteocalcin, calcitonin receptor, IL-6, and tumor necrosis factor-α (TNF-α). The primer sequences used are listed in Table 1. Briefly, total RNA from the tissue samples was extracted using TRIzol® (Invitrogen, Frederick, USA) and oligo (dT) primers were used to synthesize complementary DNA. qRT-PCR was performed using two SYBR Premix Ex Taq II (Tli RNase H Plus) (RR82LR, Takara, Ann Arbor, MI, USA) reagents on an Applied Biosystems instrument (Foster City, CA, USA) under the following cycling conditions: initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 s, annealing temperature for 15 s, and extension at 72°C for 15 s. GAPDH was used for normalization and was calculated using the $2^{-\Delta\Delta Ct}$ method.

7. Statistical analyses

All statistical analyses were performed using SPSS software (version 21, SPSS, Chicago, IL, USA). All parameters were presented as mean ± standard deviation values. All data satisfied the sphericity assumption, and the normality of the data was evaluated using the Kolmogorov-Smirnov test. The primary outcome of this study was the differences in the quality of the regenerated ridge between the three experimental groups, and the unit of analysis was the individual specimen. Repeated-measures analysis of variance (RM-

ANOVA) was used to compare the experimental groups. The Friedman test was applied to data that did not conform to a normal distribution. Results were considered statistically significant when $p < .05$ in a two-tailed test.

III. RESULTS

1. Clinical findings

There were no adverse events during wound healing at any of the experimental sites over the 8-week study period. During suture removal, it was observed that healing with primary intention had been achieved without wound dehiscence or exposure of the grafted materials.

2. Radiographic observations

After 8 weeks of healing, all experimental groups exhibited well-maintained alveolar ridge dimensions, which appeared comparable to the baseline (Fig. 3). The grafted materials were well retained inside the extraction sockets in all three groups, and only a few particles were seen scattered beyond the bony envelope (Fig. 3). Compared with the baseline, the grafted regions had higher radiopacity due to the presence of residual biomaterials. However, the grafted particles appeared to be loosely oriented in the superficial area of the graft.

3. Quantitative analysis of superimposed micro-CT images

The ridge dimensions had increased to 109.96±20.13%, 107.47±18.70%, and 104.57±17.49% of the baseline in the GBR, SB, and SB+PRFM groups, respectively (Table 1, Fig. 4), with no significant differences between the groups (p>.05).

4. Histological observations

Substantial new bone formation occurred within the sockets of all experimental groups. The regenerated alveolar ridges could be clearly distinguished from the superficial loose connective tissues by a border of new bone (Fig. 5). There was more new bone formation in the lingual and apical regions of the socket than in the buccal and coronal regions, and this bone was more mature. There were no perceptible differences in the bone formation patterns between the three groups, even in the outermost regions.

5. Quantitative/qualitative analyses in superimposed histological images

The ARAs had increased of 120.01±25.31%, 117.59±21.70%, and 114.56±25.48% of the baseline values in the GBR, SB, and SB+PRFM groups, respectively, whereas the RRAs had decreased to 93.18±29.68%, 86.90±16.44%, and 77.53±20.88% (Table 1, Fig. 5). There were no significant differences between the three groups in the quantitative analysis (p>0.05).

Qualitative analysis of the RRAs indicated that the compositions of mineralized tissue, residual biomaterials, and fibrovascular tissues did not differ significantly between the three groups (p>.05). The proportions of mineralized tissues (residual biomaterials) were 39.76±5.47% (20.69±2.17%), 37.26±10.58% (21.71±7.54%), and 39.27±8.60% (19.94±3.69%) in the GBR, SB, and SB+PRFM groups, respectively; the corresponding proportions of fibrovascular tissues were 39.55±4.86%, 41.03±6.62%, and 40.79±6.96% (Fig. 5).

6. Gene expression findings of growth factors

Several gene expression patterns were evaluated in one animal after 1 week of healing in order to assess the initial healing (Fig. 6). The expression levels of all tested growth factors (BMP-2, VEGF, TGF-ß, and FGF-2) were up-regulated in the SB and SB+PRFM groups compared with the GBR group, by 1.41- and 1.90-fold, 1.58- and 1.57-fold, 1.54-

and 1.41-fold, and 1.23- and 1.56-fold, respectively. The expression levels of osteocalcin, an osteoblast-releasing cytokine, were 1.31- and 1.72-fold higher in the SB and SB+PRFM groups, respectively, than in the GBR group. The expression levels of the transmembrane calcitonin receptor, which inhibits osteoclastic activity, were also higher in the SB and SB+PRFM groups than in the GBR group by 1.59- and 2.03-fold, respectively. Contrastingly, IL-6 and TNF-α, which are inflammatory cytokines activating osteoclastogenesis, were also up-regulated in the SB and SB+PRFM groups compared with in the GBR group by 2.04- and 1.56-fold, and 1.28- and 1.15-fold, respectively.

IV. DISCUSSION

PRF has received a lot of attention as a candidate biomaterial for alveolar ridge regeneration due to it including the release of growth factors with regenerative capacity. However, reports of its clinical effectiveness have been controversial (Pan et al., 2019). The present study compared two distinct PRF preparations, namely SB and SB+PRFM, with GBR using a particulate bone substitute and a collagen membrane for damaged extraction socket regeneration. The main findings were (i) SB and SB+PRFM produced outcomes comparable to GBR regarding bone quantity and quality after 8 weeks of healing, and (ii) using PRF increased growth factor expression for enhancing bone formation compared with conventional GBR during the first week of healing.

This study used two-wall damaged extraction socket models involving only the mesial and distal walls. Although a negative control group was not included in the experiments, a previous study using the same experimental model found that no grafting in this defect resulted in significant reductions in ridge dimensions by up to 50% (Tien et al., 2021). That study also found that placement of a particulate bone substitute with or without a collagen membrane can achieve complete preservation of the original ridge dimension; however, using a collagen membrane can help improve the quality of the regenerated ridge at the coronal area of the socket. It is plausible that using a barrier membrane could improve the outcome of socket augmentation by providing additional stability to the grafted site and preventing scattering of the grafted materials. Moreover, the barrier membrane occludes the overlying soft tissues to promote bone healing beneath the membrane. The concept of GBR is well recognized and is supported by systematic reviews (Sanz-Sanchez et al., 2015; Thoma et al., 2019). Based on this evidence, GBR was chosen as the standard-of-care reference for the present defect model and was assigned as the positive control.

In the present study, PRF was mixed with a particulate bone substitute to form the SB.

SB might be advantageous for bone augmentation procedures because of the cohesiveness of the putty-like mixture providing self-sustained stability as well as its ease of manipulation. This study found that SB and GBR produced comparable amounts of ridge augmentation, while coverage of the PRFM over SB produced no added benefits. This means that SB alone could provide sufficient stability in the two-wall defect model to match the effect of a collagen membrane and achieve complete preservation of the defect dimensions. The non-cross-linked collagen membrane is known to undergo almost complete resorption within 4–6 weeks of grafting (Rothamel et al., 2005), and PRF has been found to biodegrade after only 2–3 weeks (Gheno et al., 2021). Therefore, the role of the collagen membrane and SB is to provide stability to the grafted particles during the early stages of healing, and both treatment modalities were sufficient to achieve complete ridge preservation in the current experimental model. On the other hand, it should be noted that stability is achieved in this two-wall defect model mainly by the presence of the bone substitute particles, since a previous study demonstrated that bone grafting alone could achieve complete ridge preservation and new bone formation comparable to that with GBR (Tien, Lee et al. 2021). Therefore, based on the data obtained in this study as well as that reported in the literature, it is difficult to determine whether there are differences in dimensional stability between the GBR, SB and SB+PRF conditions.

Gene expression analysis performed after 1 week of healing in this study revealed greater quantities of favorable signaling molecules in the PRF groups than in the GBR group, which is consistent with findings in the literature. PRF consists of a dense fibrin network that mostly traps platelets and leukocytes, which slowly releases growth factors and has the advantage of promoting the regeneration of hard and soft tissue (Dohan Ehrenfest et al., 2009; El Bagdadi et al., 2019; Miron et al., 2017). Growth factors are mediators that regulate cellular activities such as proliferation, chemotaxis, differentiation, and angiogenesis to initiate the healing response. Notably, the expression of BMP-2, which is a potent osteogenic inducer that differentiates stem cells into osteoblasts, was almost two-fold higher in the PRF groups than in the GBR group. Subsequently, the expression of

IL-6, which is activated by osteoblasts and suppresses osteoclast activity, was also 1.5-fold higher in the PRF groups. These findings are in agreement with those of a recent clinical trial showing that the application of PRF to extraction sockets resulted in increased local concentrations of growth factors for up to 1 week (Wang, Fok et al. 2022). However, the enhanced growth factor expression levels in that study did not translate into improved clinical outcomes when compared with natural socket healing.

Since PRF has been described as inducing the release of growth factors that promote bone formation, higher quality regenerated ridges would have been expected in the PRF groups of this study. However, the mineralized proportions of the regenerated ridges did not differ significantly between the PRF and GBR groups after 8 weeks of healing, and the bone formation pattern was also the same in these groups. Possible explanations for these findings are the following: (i) the amount of growth factors released from PRF was insufficient to induce a significant biological effect on bone formation and maturation, and (ii) the experimental site had sufficient potential to heal within 8 weeks using GBR without help from additional growth factors. In this study, the PRF groups exhibited up-regulation of the genes related to bone proliferation compared with the GBR group after 1 week. However, a previous histological study involving dogs showed woven bone formation after 14 days of healing in an intact extraction socket, which continued to mature until 4–6 months (Cardaropoli et al., 2003). The results regarding the quality of the regenerated ridge might therefore be considered inconclusive. The beneficial effects of PRF might occur at an earlier stage of healing or only in more challenging defect configurations.

There were some limitations in this study. Firstly, an ideal experimental design should include a negative control group, which was not the case in this study. However, since the first premolar has a single root, the number of experimental sites was limited to three. Considering that experimental conditions can be better controlled by confining the group allocation to the same side of the mandible, a negative control group was excluded. Instead, a positive control group with GBR was hypothesized to provide more useful and interesting

information than a negative control; moreover, outcomes of empty and bone-graft-only groups have been demonstrated in previous studies (Lee et al., 2015; Tien et al., 2021). Secondly, gene expression was measured in only one specimen at a specific time point. Collecting larger numbers of samples over extended healing periods would better clarify the relation between the gene expression and histological outcomes. In addition, the absence of a control group means that the baseline gene expression profile in the spontaneously healing socket was also lacking. Therefore, we may only assume that gene expression levels at the PRF sites were above physiological levels, which was addressed in our hypothesis as part of the scientific rationale behind PRF use. Nonetheless, a previous in vivo study found that GBR sites exhibited similar or even higher concentrations of growth factors compared with the negative control site (An, Strauss et al. 2021), which verifies our assumption. Also, it must be mentioned that the aim of the present study was to compare the regenerative capacity of the two PRF preparations with GBR rather than to validate the current PRF system and identify definitive correlations between gene expression profiles and regenerative outcome, for which the sample would have been too small (n=1). Thirdly, the alveolar bone dimensions around the mesial roots of the corresponding distal experimental sites were used as the pre-extraction baseline reference for measuring post-operative dimensional changes. Dog mandibles tend to widen towards the posterior aspect; however, in the premolar sites, the difference between the width of mesial and distal root areas of the same tooth were considered negligible (Fig. 3). Also, the sites were rotationally allocated to the groups to account for the differences in healing potential.

In summary, SB consisting of PRF and DPBM can regenerate the original ridge dimensions of two-wall damaged extraction sockets as effectively as GBR using DPBM and a non-cross-linked collagen membrane. Although PRF in combination with DPBM seemed to enhance the expression levels of growth factor during the first week of healing, the histological bone quality after 8 weeks was comparable to that for GBR.

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TABLES

Table 1. Results for dimensional alterations in radiographic and histometric (augmented ridge and regenerated ridge) analyses of the three experimental groups: (i) guided bone regeneration (GBR), in which deproteinized porcine bone mineral was grafted and covered with a noncrosslinked collagen membrane, (ii) sticky bone substitute (SB) with a platelet-rich fibrin membrane (PRFM), and (iii) SB alone. Augmented ridge implies the entire grafted area and regenerated ridge implies the area containing newly formed bone within the augmented ridge.

	GBR		SB		SB+PRFM		*p*
Radiography (augmented ridge)	109.96±18.70	[90.33–129.59]	107.47±17.49	[89.11–125.83]	104.57±20.13	[83.45–125.69]	0.090
Histology (augmented ridge)	120.01±25.30	[93.45–146.57]	117.59±21.70	[94.83–140.36]	114.56±25.48	[87.82–141.29]	0.176
Histology (regenerated ridge)	93.18±29.68	[62.03–124.33]	86.90±16.44	[69.65–104.16]	77.53±20.88	[55.61–99.44]	0.088

Note. Values represent dimensional percentages (augmented ridge or regenerated ridge) of the area of the alveolar ridge at the experimental site relative to the pre-extraction baseline. Data are presented as mean±standard-deviation or [95% confidence interval] values.

FIGURES

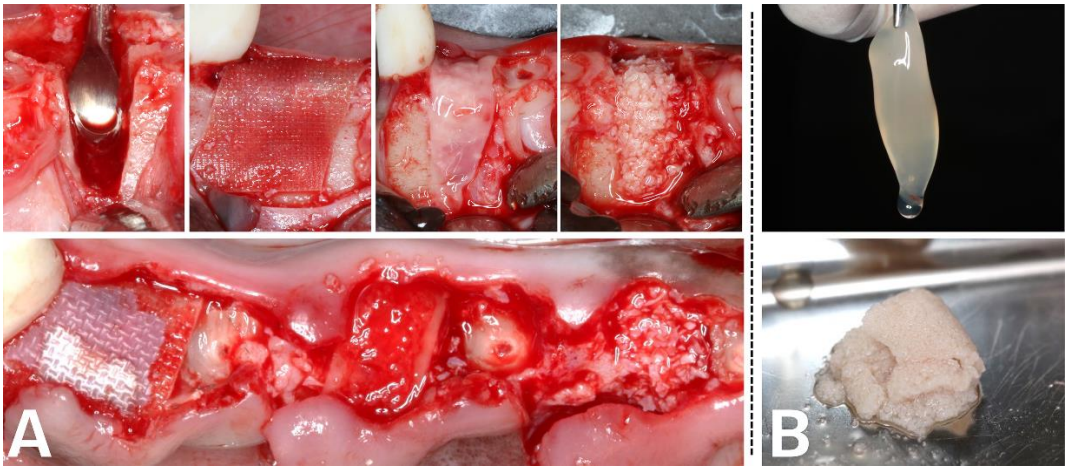

Figure 1. Experimental procedures. **(A)** After extracting the distal root of the second, third, and fourth mandibular premolars, the buccal and lingual walls were removed (first upper image). The defects were randomly allocated to three experimental groups: (i) guided bone regeneration (GBR), in which deproteinized porcine bone mineral (DPBM) was grafted and covered with a noncrosslinked collagen membrane (second upper image), (ii) sticky bone (SB) substitute with a platelet-rich fibrin (PRF) membrane (PRFM) (third upper image), and (iii) SB alone (fourth upper image). The three groups were produced along the unilateral mandible, and the mesial roots were decoronated and preserved (lower image). **(B)** Preparation of SB. Injectable PRF, in which liquid-type PRF (upper image) was mixed with DPBM to form a putty-like mixture (lower image).

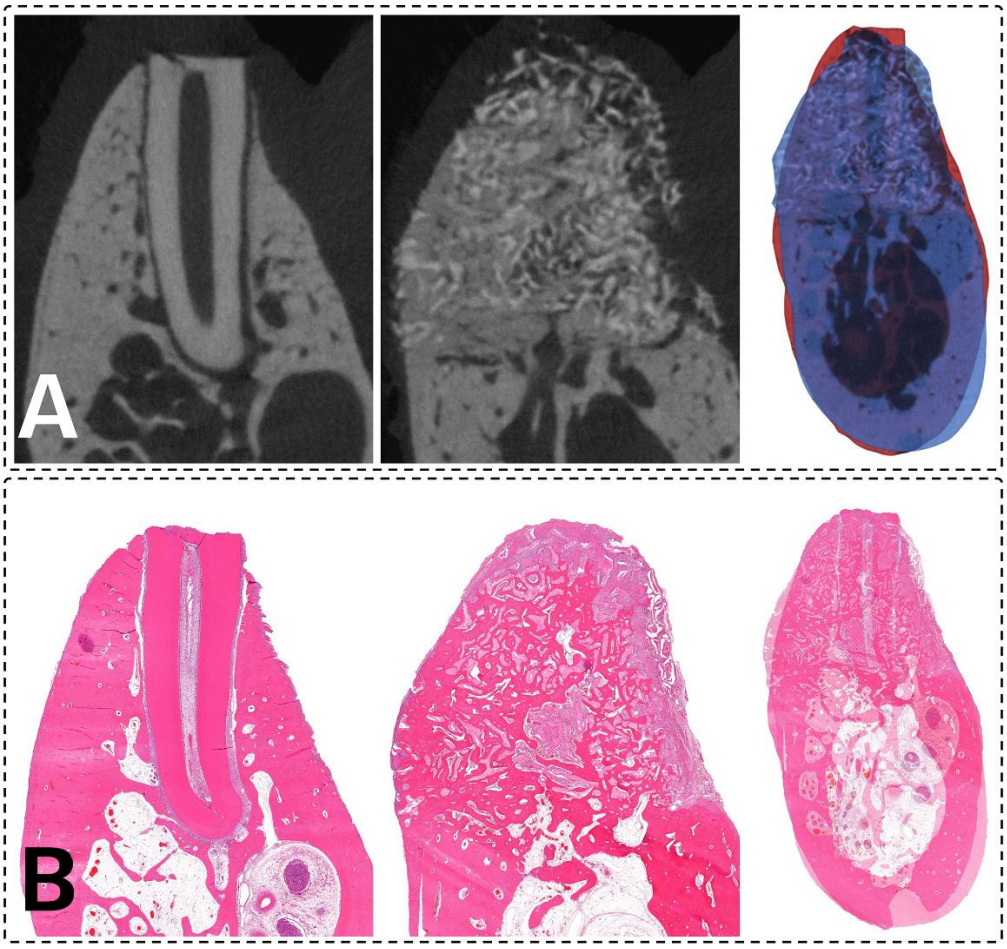

Figure 2. Ridge dimension around the mesial root used as the baseline reference (left images) for measuring dimensional changes at the corresponding distal experimental sites (middle images) after 8 weeks of healing. The two sites were superimposed (right images) to measure the percentage changes in the augmented ridge area (ARA) using microcomputed tomography (micro-CT) **(A)** and on histological sections **(B).**

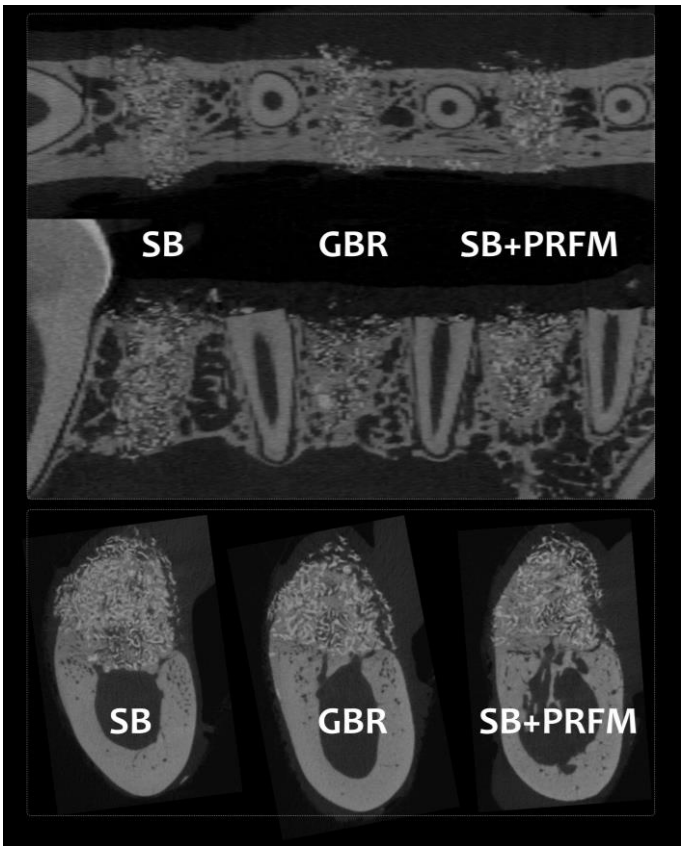

Figure 3. Microcomputed tomography views of the three groups allocated at each experimental site. The top and middle images show axial and panoramic views of the three experimental sites and pristine sites of the unilateral mandible. The bottom images show cross-sectional views of the experimental sites. All experimental sites showed preserved ridge dimensions compared with the respective pristine sites at the mesial root.

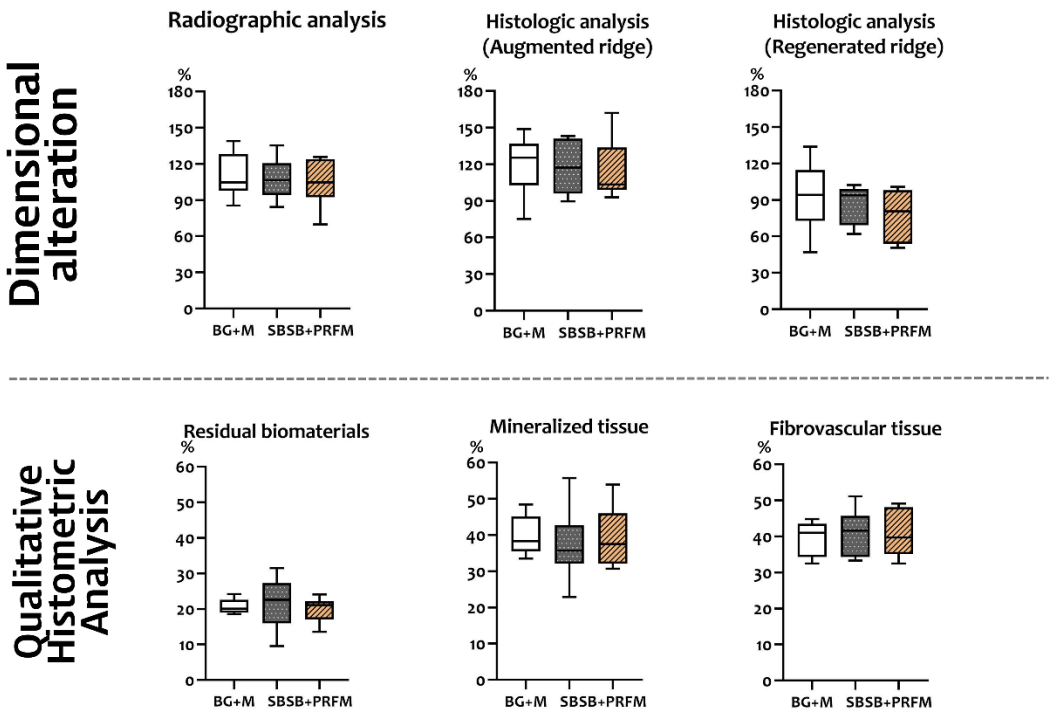

Figure 4. Box plots showing the dimensional alterations from baseline (pre-extraction) and 8 weeks after alveolar ridge preservation (upper) at the experimental sites, and the results of qualitative histological analyses (lower) of the three experimental groups. There was no significant differences in either dimensional or qualitative parameters among the three groups.

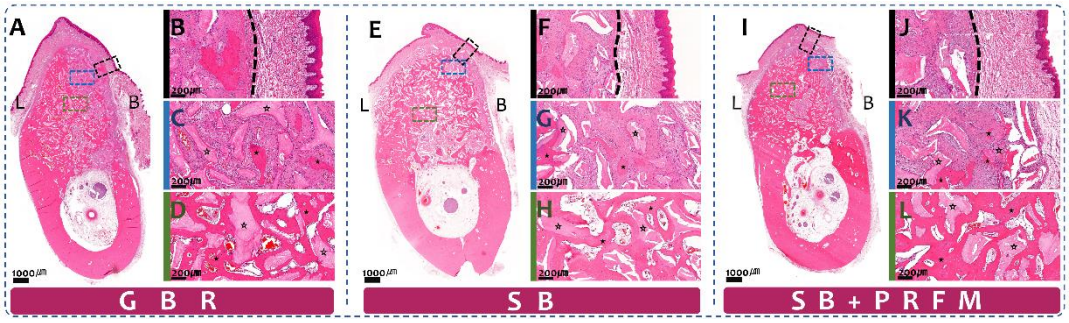

Figure 5. Histological sections of the three experimental groups at high and low magnifications after staining with hematoxylin and eosin. **(b, f, j)** The regenerated ridges could be distinguished from the outer connective tissues by a border of new bone. There was more new bone formation in the lingual (L) and apical regions of the socket **(d, h, l)** than in the buccal (B) and coronal regions **(c, g, k),** and this bone was more mature.

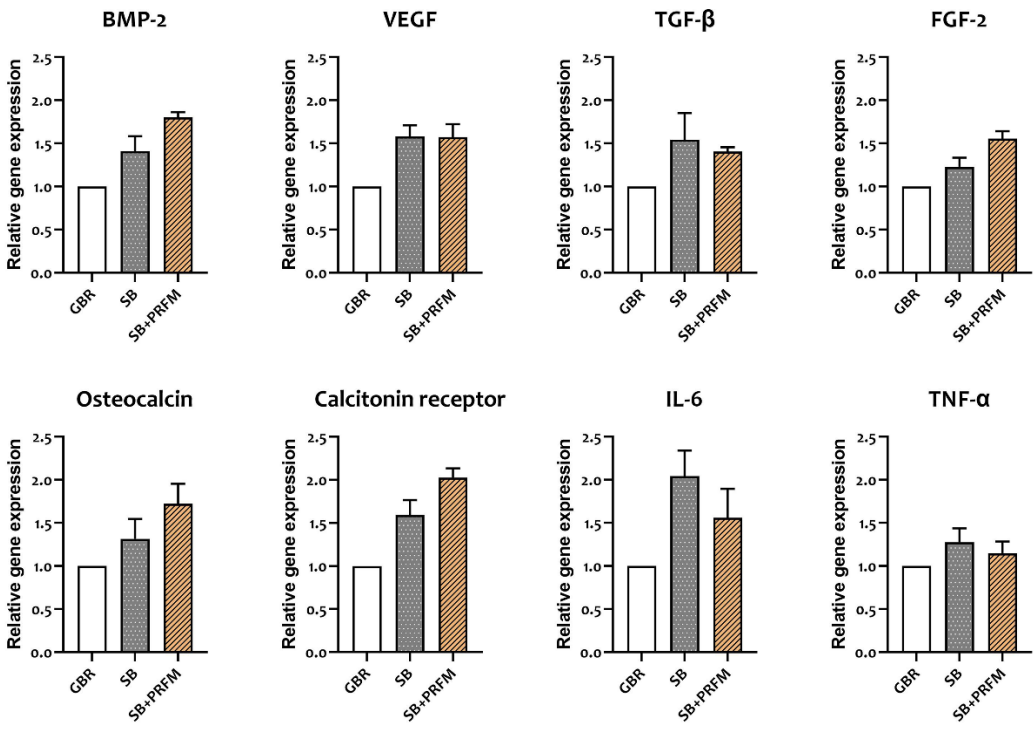

Figure 6. Box plots comparing the gene expression of bioactive factors from quantitative reverse-transcription polymerase chain reactions between the three experimental groups at 1 week. Gene expression levels of all growth factors were higher in the SB and SB+PRFM groups than in the GBR group. Data are presented as mean and standard-deviation values.

국문요약

파괴된 발치와 처치 시 혈소판이 풍부한 피브린(PRF)의 골재생 효과

<지도교수 **이 중 석**>
연세대학교 대학원 치의학과
홍 규 진

혈소판이 풍부한 피브린(PRF)은 혈소판과 백혈구로 이루어진 섬유기질로 구성되며, 상처 치유와 조직의 회복에 필수적인 다양한 성장인자들을 방출하여 치과 영역의 여러 가지 술식에 사용되었다. 특히 발치 후 치조제 보존을 위해 발치와에 PRF 를 이식했을 때, 방사선학적으로 확인되는 골 충전을 보고한 선행 연구들이 있었으나 가장 최근의 한 선행 연구는 PRF 자체가 발치 후 치조제의 흡수를 감소시키는 데 유의미한 영향은 없다고 하였다. 이전의 여러 발치와 모델을 사용한 연구에서 다양한 결손부의 형태와 골이식재, 차폐막의 사용 여부 등에 따른 치조제 보존 정도를 평가한 바 있다. 본 연구에서는 골이식을 위한 생체 재료에 PRF 를 추가하면 기존의 골유도재생술 보다 체적 안정성 및 새롭게 형성된 신생골의 질이 향상될 것이라는 가정을 전제로, 두 가지 형태의 PRF 를 탈단백화 된 돼지 유래 골 대체제(Deproteinized porcine bone mineral, DPBM)와 함께 사용하여 파괴된 발치와에서 골재생의 효과를 확인하고자 했다.

7 마리 성견의 하악 편측의 제 2, 3, 4 소구치를 편측 절단하고 원심 치근을 발거한 후, 협측 및 설측 골벽을 외과적으로 삭제하여 2 벽성 골

결손부를 형성했다. 대조군(GBR 군)에는 DPBM 과 흡수성 콜라겐 차폐막을 사용하여 골유도재생술을 시행하고, 실험군 1(SB 군)에는 액체형 PRF 와 DPBM 을 혼합하여 제작한 점착성이 있는 골대체제(Sticky bone, SB)를 이식하였으며 실험군 2(SB+ PRFM 군)에는 SB 를 이식 후 고체형 PRF 를 압착하여 제작한 PRF 차폐막 (PRF membrane, PRFM)을 피개하였다. 1 주일 후 PRF 의 성장인자 방출 효과를 평가하기 위해 한 개체의 표본을 대상으로 정량적 역전사 중합효소 연쇄 반응을 시켰다. 나머지 6 마리의 개체는 치유 8 주 후에 방사선학적, 조직학적 분석을 시행했다. 치근을 발거하지 않은 근심측과 치조제 보존술을 시행한 원심 측의 방사선 및 조직 영상을 중첩하여 치조제의 면적 변화량을 계측하였다. 실험군과 대조군은 모두 Kolmogorov-Smirnov 검정에서 정규 분포를 따랐으며, 방사선학적 및 조직학적 계측치의 통계적 비교는 Repeated-mesures ANOVA 와 Friedman 검정으로 시행하였다 ($p<0.05$).

8 주간의 치유 기간 동안 모든 개체는 특별한 합병증 없이 양호한 치유를 보였다. 방사선학적 및 조직학적 계측 결과, 근심 측 치조제 면적에 대한 실험 부위의 치조제 면적의 변화량은 세 군 간에 유의한 차이가 확인되지 않았다 (109.96±20.13%, 107.47±18.70%, 104.57±17.49%). 마찬가지로 결손 부위의 신생골 형성의 정도도 모든 실험군에서 유사하게 나타났다. 술후 1 주에서, PRF 를 사용한 실험군에서는 대조군 보다 조골세포 및 파골세포 마커인 오스테오칼신(Osteocalcin) 및 칼시토닌 수용체(Calcitonin receptor)의 발현이 증가되었다. 또한 혈관신생 및 염증 마커인 혈관내피세포성장인자(Vascular endothelial growth factor, VEGF)와 인터루킨 6(Interleukin 6, IL-6)도 약간 증가시켰다.

결론적으로 PRF 와 DPBM 을 혼합한 SB 의 사용은 파괴된 발치와의 치조제 보존에 있어 비가교성 흡수성 콜라겐 차폐막을 사용한 기존의 GBR 술식과 유사한 재생 효과를 얻을 수 있다.

핵심되는 말: 동물실험, 치과 임플란트, 조직학, 분자생물학, 치료