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The adjunctive effect of polydeoxyribonucleotide on bone formation in alveolar ridge preservation: A pre-clinical in vivo study

Young-Chang Ko[1] | Jungwon Lee[1,2] | Istvan Urban[3,4,5] | Yang-Jo Seol[1] | Yong-Moo Lee[1] | Ki-Tae Koo[1]

[1]Department of Periodontology, School of Dentistry and Dental Research Institute, Seoul National University, Seoul, Republic of Korea

[2]One-Stop Specialty Center, Seoul National University Dental Hospital, Seoul, Republic of Korea

[3]Division of Periodontology, Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, Boston, Massachusetts, USA

[4]Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, Michigan, USA

[5]Department of Periodontology, University of Szeged, Szeged, Hungary

Correspondence
Jungwon Lee and Ki-Tae Koo, Department of Periodontology and Dental Research Institute, School of Dentistry, Seoul National University, 101, Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea.
Email: houy1980@snu.ac.kr and periokoo@snu.ac.kr

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Abstract

Aim: This study investigated the adjunctive effect of polydeoxyribonucleotide (PDRN) on bone formation in alveolar ridge preservation (ARP) sockets.

Materials and Methods: Both mandibular second, third and fourth premolars of eight beagle dogs were randomly divided into ARP and ARP/PDRN groups. Following tooth extraction, ARP procedures were conducted using collagenized alloplastic graft material and bilayer collagen membrane soaked with normal saline (ARP group) or PDRN (ARP/PDRN group) for 10 min before application. Both groups were also randomly allocated to 2-, 4- or 12-week healing subgroups. The primary endpoint of this study was to compare histomorphometric differences between ARP and ARP/PDRN. The secondary endpoints of this study were to compare micro-CT analysis and three-dimensional volumetric measurement between the two groups.

Results: In the histomorphometric analysis, the ARP/PDRN group exhibited greater new bone formation at coronal, middle and total position compared with the ARP group at 2-week healing. The number of newly formed blood vessels was higher in the ARP/PDRN group than in the ARP group at 2- and 4-week healing. In micro-CT analysis, the mean new bone volume/total bone volume between ARP and ARP/PDRN was statistically significant at 2-week healing. Ridge volume alterations were significantly decreased in the ARP/PDRN group during entire healing time compared with the ARP group, especially on the buccal side.

Conclusions: The application of PDRN in ARP might provide additional benefits for early bone regeneration and maintenance of buccal ridge volume.

KEYWORDS
alveolar ridge preservation, bone regeneration, polydeoxyribonucleotide

Clinical Relevance

Scientific rationale for study: Polydeoxyribonucleotide (PDRN) has been shown to promote cell proliferation, angiogenesis, anti-inflammatory effects and tissue repair. This study aimed to evaluate the clinical feasibility of PDRN in the bone regeneration process.

Principal findings: PDRN enhanced new bone formation in alveolar ridge preservation sockets and prevented significant ridge volume reduction on the buccal side. The angiogenic effect of PDRN may provide an additional benefit in bone healing; however, further research is required.
Practical implications: PDRN has the potential for improved bone regeneration and maintenance of alveolar ridge volume in alveolar ridge preservation as an adjunctive therapeutic agent.

1 | INTRODUCTION

Tooth extraction is a common dental procedure necessitated by various pathologies, such as periodontitis, dental caries and tooth fractures. Following extraction, the socket undergoes remodelling through a series of physiological processes, resulting in horizontal and vertical dimensional alterations of the alveolar ridge (M. G. M. G. Araujo & Lindhe, 2005; Cardaropoli et al., 2003; Trombelli et al., 2008). In humans, these dimensional changes in the alveolar ridge after tooth extraction have been reported to average 3.87 mm horizontally and 1.67 mm vertically (Van der Weijden et al., 2009). Due to the atrophic reduction in ridge volume following tooth extraction, placing an implant in the optimal position can be a clinical challenge, often requiring additional surgical intervention, such as guided bone regeneration. Implant placement in the optimal position is crucial for the long-term success of the implant, as it allows proper prosthesis design and oral hygiene maintenance (Chen et al., 2023; Schwarz et al., 2018). Due to these clinical demands, alveolar ridge preservation (ARP) is often performed before implant placement.

ARP is a predictable procedure introduced in the 2011 Osteology Consensus Report (Hammerle et al., 2012) and has become the most widely used technique in implant treatment to minimize the dimensional changes in the alveolar ridge following tooth extraction (M. Araujo et al., 2008; M. G. Araujo & Lindhe, 2009; Avila-Ortiz et al., 2019). Tonetti et al. (2019) reported that the ARP procedure can prevent ridge dimensional alterations by as much as 1.5–2.4 mm horizontally, 1–2.5 mm vertically in the mid-buccal region and 0.8–1.5 mm in the mid-lingual region (Tonetti et al., 2019). The ARP procedure also offers additional clinical benefits, such as maintaining the aesthetic ridge contour for prosthetic restoration and simplifying the implant surgery procedure (Hammerle et al., 2012; Vignoletti et al., 2012). However, several studies have shown that residual bone substitutes in ridge preservation site might interfere with wound healing of the alveolar ridge, suggesting that additional healing-promoting factors are needed (Adams, 2022; Carmagnola et al., 2003).

Polydeoxyribonucleotide (PDRN) is a compound composed of short deoxyribonucleotide polymers extracted from salmon sperm, and it is widely used in various medical fields such as aesthetics, wound healing and tissue regeneration for diabetic ulcer and thermal injury. PDRN is well known for promoting cell proliferation, angiogenesis and anti-inflammatory effects through its interaction with the A2 purinergic receptor (Galeano et al., 2008; Squadrito et al., 2017). In dental applications, Bitto et al. (2013) conducted a study on experimental periodontitis in rats and reported that PDRN reduced inflammation and apoptotic molecules in periodontitis lesions (Bitto et al., 2013). Moreover, recent studies have demonstrated the bone regenerative capacity of PDRN (D. S. Kim, Lee, et al., 2021; S. K. Kim et al., 2016), and our previous pre-clinical studies also confirmed that PDRN enhanced early bone formation in the sinus augmentation or lateral bone augmentation (D. Lee et al., 2022; D. Lee et al., 2023). However, it remains uncertain whether the application of PDRN will be effective and clinically beneficial for bone regeneration at ARP procedure.

The primary endpoint of this study was to compare histomorphometric differences between ARP only and ARP with PDRN using collagenized alloplastic graft material and a collagen membrane. The secondary endpoints of this study were to compare micro-CT analysis and three-dimensional volumetric measurement between the two groups.

2 | MATERIALS AND METHODS

2.1 | Animals

This animal study was approved by Seoul National University Institutional Animal Care and Use Committee (SNU-220502-3-2). Eight male beagle dogs, aged 1–2 years and weighing 10–12 kg, were utilized in this study. All experimental procedures were conducted in accordance with the Animal Research: Reporting of In Vivo Experiments guidelines 2.0 (Percie du Sert et al., 2020). All animals were individually housed in separate cages, maintained at an ambient room temperature of 23 ± 2°C, with a relative humidity of 50 ± 10%, a 12-h light/dark cycle, and air conditioning providing 12–18 changes per hour. Following surgery, a soft diet (HappyRang, Seoulfeed Company, Gyeonggi-do, Korea) was administered to the animals.

2.2 | Sample size calculation

In our previous studies, we examined the regenerative effects of PDRN on sinus augmentation or lateral augmentation with a significant increase in new bone formation (D. Lee et al., 2022, 2023). However, the effect of PDRN in ARP has not been fully investigated. We determined a mean difference of ≥13% and a standard deviation of 8% for this study with a 5% α and 80% power (effect size [*d*], 1.625) for the superiority analysis. The null hypothesis (H0) was that there was no difference between ARP and ARP with PDRN, while the alternative hypothesis (H1) indicated that there was a difference between the two groups. To calculate the sample size, we used a software program (G*Power, Heinrich Heine University, Düsseldorf, Germany). Finally, eight animals were decided for this study.

2.3 | Surgical protocol

The experimental outline and surgical procedures are illustrated in Figure S1. In accordance with single-blinded simple randomization protocol and split-mouth design, the experiment was performed. Second, third or fourth premolars at one side of mandible were allocated to 2-, 4- or 12 weeks of healing following ARP. The premolars at one side of the mandible allocated as the ARP group (alloplast + collagen membrane only), and premolars at contralateral side were determined as the ARP/PDRN group (PDRN with alloplast + collagen membrane). The timeframe assigned to each premolar was ensured to be symmetrical on both sides of the mandible (Table S1). To evaluate the effect of PDRN at each phase of bone regeneration, the two groups were further divided into three subgroups with healing periods of 2, 4, and 12 weeks. Prior to the surgical procedures, general anaesthesia was induced using an intravenous injection of 0.1 mg/kg tiletamine/zolazepam (Zoletil, Virbac, Carros, France), 2.3 mg/kg xylazine (Rompun, Bayer Korea, Ansan, Korea) and 0.05 mg/kg atropine sulphate (Jeil, Daegu, Korea). Additionally, local anaesthesia was administered with 1:100,000 epinephrine-containing lidocaine (Huons Co. Ltd., Seongnam, Korea). Each premolar was hemisected using a diamond bur, and the mesial root was extracted. Root canal treatment was performed on the remaining distal root, and the canal was sealed with a hydrosoluble calcium hydroxide paste (CleaniCal; Maruchi, Gangwon, Korea) and intermediate restorative material (IRM; Dentsply Sirona, Milford, DE, USA) (J. Lee et al., 2019). In the ARP group, the ARP procedure was carried out on the mesial extraction socket using a collagenized alloplastic graft material (60% hydroxyapatite + 40% β-tricalcium phosphate) (Osteon 3 Collagen Cylinder type; 0605, Genoss, Gyeonggi-do, Korea) and a bilayer collagen membrane (Collagen graft 2, PSD10C, Genoss, Gyeonggi-do, Korea) with normal saline. In the ARP/PDRN group, prior to application, the alloplastic graft material was soaked in 1 mL of PDRN (1.875 mg/mL, Genoss, Gyeonggi-do, Korea) for 10 min, and the collagen membrane was treated with 2 mL of PDRN (1.875 mg/mL) for 10 min. The surgical site was sutured with 5/0 monosyn (B. Braun, Melsungen, Germany) using a hidden X technique (Park et al., 2016). After the surgery, intravenous antibiotics (cefazolin, 20 mg/kg) and analgesics (tramadol HCl, 5 mg/kg) were injected in order to relieve post-operative pain and inflammation. Radiographs were taken at each surgery, and intra-oral scans and professional plaque control were conducted every 2 weeks during the experiment.

2.4 | Retrieval of specimens

Following the surgical protocol, eight animals were euthanized by administering potassium chloride (Jeil, Daegu, Korea) through the carotid artery. Specimens were collected from the dissected mandible and subsequently fixed in a 10% formaldehyde solution for a period of 2 weeks.

2.5 | Outcome variables

The primary outcome of this study was the amount of histological new bone formation in ARP sockets. Secondary outcomes were clinical findings and radiographic new bone volume in ARP sockets from micro-CT analysis. 3D intra-oral scan data analysis was also conducted to investigate the alteration of alveolar ridge volume following ARP procedure.

2.6 | Micro-CT analysis

Micro-CT scans of specimens at ARP sockets were performed using a micro-CT device (SkyScan-1173, Kontich, Belgium), and the resulting two-dimensional micro-CT images were saved as 2240 × 2240 pixel BMP files. The specific exposure conditions during the micro-CT scans included a voltage of 92 kV, a current of 120 mA, an exposure time of 500 ms, a rotation step of 0.3°, frame averaging of 4, a 1.0-mm aluminium filter and an image pixel size of 19.18 μm. Subsequently, the obtained images were processed and reconstructed into 3D images using CTVox software (Bruker, Kontich, Belgium), and the volume of each structure was measured using CTAn software (Bruker, Kontich, Belgium).

Volumes of interest (VOIs) were determined by collecting round-shaped regions of interest (ROIs) on horizontal section images, according to the boundaries of each extraction socket. The volume of new bone, residual bone graft particle and total bone within the VOI was measured using separate 8-bit greyscale thresholds (Song et al., 2020). The greyscale range for new bone volume (NBV), residual bone graft particle volume (RBV) and total bone volume (TBV) was set by an experienced and calibrated examiner (Y.C.K.) with repeated measurements. As a result, the greyscale range of this study was determined as 45–69 for new bone, 70–255 for residual bone graft particle and 45–255 for total bone. The results were presented as the percentage of new bone and residual bone graft particle volume relative to the TBV.

2.7 | Histological analysis

Histological preparation procedures were conducted with reference to previous studies (J. J. Kim et al., 2019; Ko et al., 2023). Tissue blocks were obtained from mandibular segment specimens and demineralized in a 12.5% EDTA solution for 4 weeks. Subsequently, the tissue blocks were embedded in paraffin wax and sectioned using a microtome (HM315, Microme, Walldorf, Germany) into approximately 3-μm-thick slices along the bucco-lingual direction and parallel to the long axis of the tooth. Two sections from the central area were stained with haematoxylin–eosin (H&E) and Masson's trichrome solution for histological analysis. Histological observations were conducted using a light microscope (DP72; Olympus, Tokyo, Japan), and histological images were captured as digital files with a digital imaging system (DP Controller; Olympus).

Histomorphometric analysis was conducted using digital image software (ImageJ, National Institutes of Health, Bethesda, MD, USA). A square-shaped ROI was used, and the size of ROI was arbitrarily determined to be 1.5 × 1.5 mm^2, considering the size of ARP sockets. Within ARP sockets, ROIs were designated at the uppermost part

from the bone crest (coronal), the lowermost part from the root apex (apical) and the centre of the two ROIs (middle). The area of new bone (NB), fibrovascular connective tissue (FCT) and residual bone graft particles (RGP) were semi-automatically measured and presented as the proportion (%) of each structure relative to the area of the ROI. Additionally, the number of newly formed blood vessels within the ROI was examined to assess the angiogenic effect of PDRN during bone healing. In order to discriminate the blood vessels from other structures, the newly formed blood vessels were arbitrarily defined by the circularity range of 0.30–1.00 and the size of 150–1500 pixels using ImageJ software, and counted within the same ROIs at coronal, middle and apical positions of ARP area.

2.8 | Three-dimensional volumetric measurement

Volumetric evaluations at surgical sites were conducted with reference to previous studies (Ben Amara et al., 2019, 2021; Schmitt et al., 2016). Intra-oral scans were performed every 2 weeks using an optical oral scanner and its accompanying software (Medit i500, Medit Corp., Seoul, Korea), with the data stored as stereolithography (STL) files. The two sets of three-dimensional (3D) data to be compared were processed and automatically superimposed using a 3D geometric software program (GOM Inspect, GOM, Braunschweig, Germany).

The measurements of volumetric deviation between two sets of 3D data were conducted on both the buccal and lingual sides of ARP sockets, using the extension line of central grooves as a reference. The ROI was identified on the surface of the 3D geometric data, and the width of the ROI was determined based on the mesio-distal distance of the pre-existing mesial root. The vertical boundary of the ROI was positioned 3 mm from the gingival margin of the adjacent distal root. The volumetric change (mm^3) between the two sets of 3D data was automatically calculated as integrated linear deviation values (mm). To minimize potential errors that could arise during intra-oral scanning, linear deviation values outside the range of −3 to 3 mm were excluded from this study. Furthermore, to examine the volumetric changes over time, comparisons were made at 4 (T4), 8 (T8) and 12 (T12) weeks of healing using the 3D data at baseline (0 weeks, T0) (T4–T0, T8–T0 and T12–T0).

2.9 | Statistics

Statistical analysis was conducted using a commercially available software (GraphPad Prism, CA, USA), and all data were presented as median, quartiles, maximum, minimum, mean and standard deviation. Due to the small sample size, non-parametric analysis was conducted. To compare the differences between the ARP and ARP/PDRN groups in each radiographic, histomorphometric and volumetric measurement, Wilcoxon matched-pairs signed-rank test was performed, considering split-mouth design. A p-value of less than .05 was considered statistically significant.

3 | RESULTS

3.1 | Clinical findings

Throughout the entire experimental period, none of the animals ($n = 8$) exhibited any severe post-operative complications. All surgical sites healed uneventfully, without any signs of inflammation, and displayed complete soft tissue wound closure as well as favourable bone formation at all ARP locations.

3.2 | Micro-CT analysis

The results of the micro-CT evaluation can be seen in Figure 1 and Table S2. The mean and standard deviations of NBV/TBV values were 10.84 ± 2.51% versus 14.29 ± 3.82% in 2-week healing (subgroup 1) (ARP vs. ARP/PDRN), showing statistically significant differences ($p = .0078$). Meanwhile, the mean NBV/TBV values were 22.44 ± 1.20% versus 24.64 ± 5.25% in 4-week healing (subgroup 2) and 29.90 ± 3.78% versus 31.85 ± 6.40% in 12-week healing (subgroup 3) (ARP vs. ARP/PDRN). There were

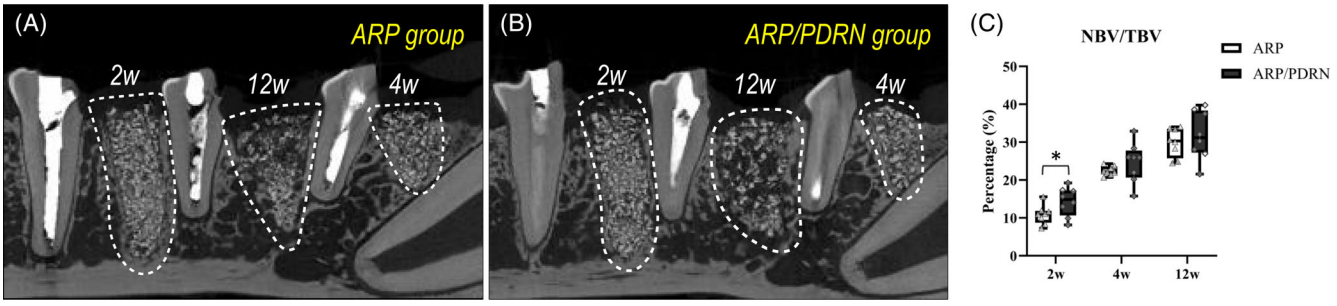

FIGURE 1 Micro-CT images (a, b) and box and whisker plot of the percentages of new bone volume relative to total bone volume (c) in the ARP group and the ARP/PDRN group (Bar = 3 mm). Hashed lines outline grafted sockets. ARP, alveolar ridge preservation; NBV, new bone volume; PDRN, polydeoxyribonucleotide; TBV, total bone volume. *$p < .05$.

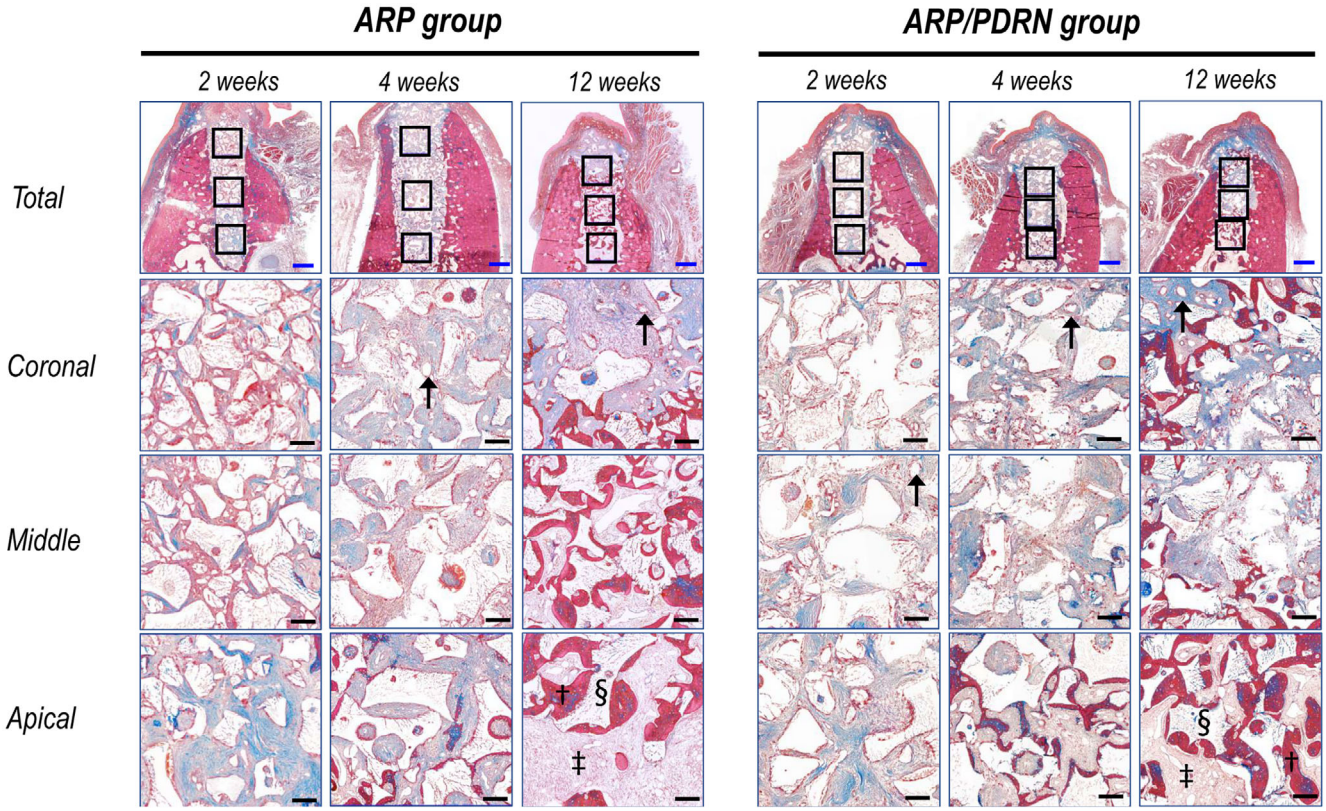

FIGURE 2 Representative images of Masson's trichrome staining in ARP and ARP/PDRN group (Blue bar = 1000 μm; black bar = 200 μm). †New bone (NB); ‡fibrovascular connective tissue (FCT); §residual bone graft particle (RGP); black arrow, newly formed blood vessel. ARP, alveolar ridge preservation; PDRN, polydeoxyribonucleotide.

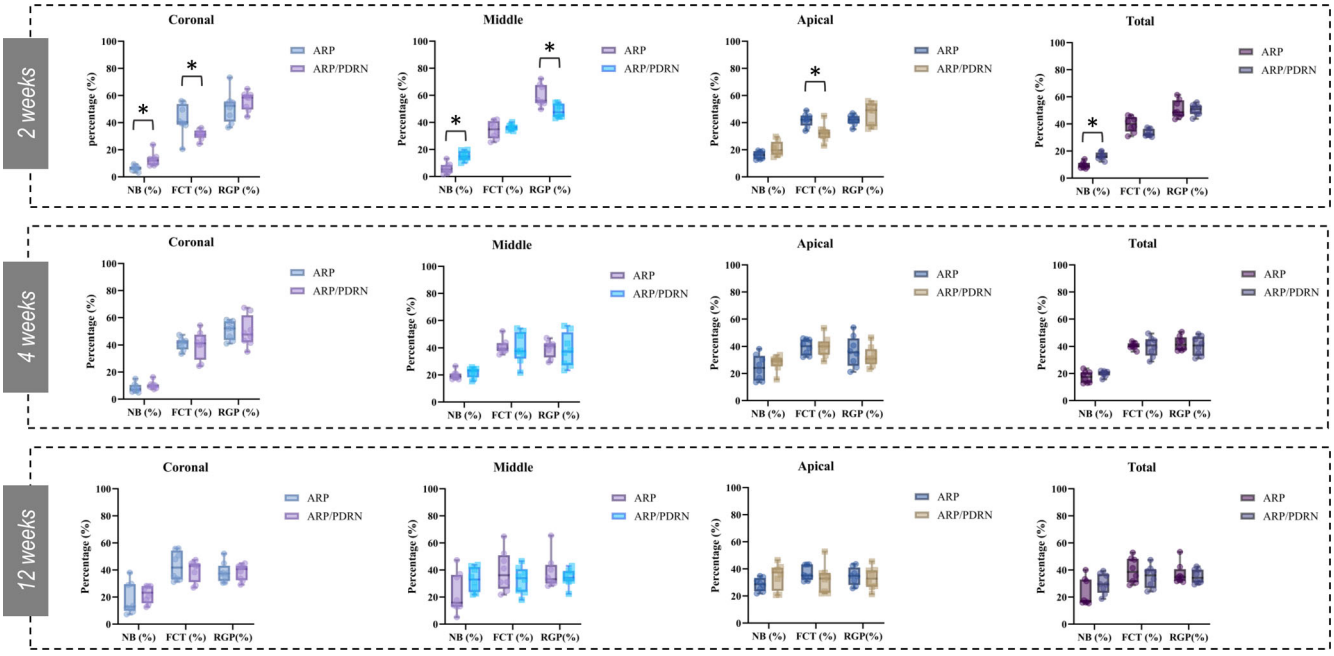

FIGURE 3 Box and whisker plot of histomorphometric results at coronal, middle and apical positions in the ARP and ARP/PDRN groups. ARP, alveolar ridge preservation; FCT, fibrovascular connective tissue; NB, new bone; PDRN, polydeoxyribonucleotide; RGP, residual bone graft particle. *$p < .05$.

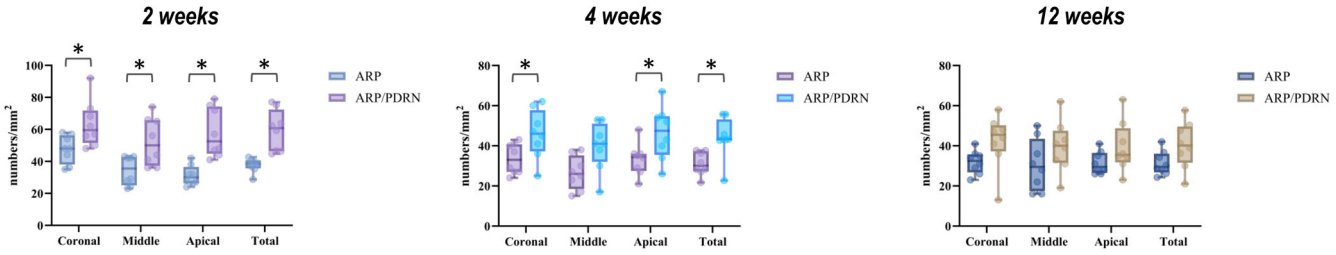

FIGURE 4 Box and whisker plot of the number of newly formed blood vessels within region of interest at coronal, middle and apical positions in the ARP and ARP/PDRN groups. *$p < .05$. ARP, alveolar ridge preservation; PDRN, polydeoxyribonucleotide.

no statistically significant differences between two groups ($p = .3125$ in 4-week healing, and $p = .2500$ in 12-week healing, respectively).

3.3 | Histomorphometric analysis

In the histomorphometric analysis, the ARP/PDRN group exhibited greater new bone formation at coronal, middle and total position compared with the ARP group at 2 weeks (Figures 2 and 3, S2 and Table S3). The mean percentages of new bone area (ARP vs. ARP/PDRN group, respectively) were 6.38 ± 1.87% versus 12.86 ± 5.05% at the coronal position, 6.02 ± 3.88% versus 15.22 ± 3.45% at the middle position and 9.49 ± 2.41% versus 16.29 ± 2.60% at the total position in 2-week healing, showing statistically significant differences ($p = .0078$, .0156, and .0156). However, there were no significant differences at the apical position between two groups in 2-week healing. In addition, in 4 and 12 weeks of healing subgroups, no statistically significant differences between the two groups were observed at coronal, middle, apical and total positions.

Meanwhile, a higher number of newly formed blood vessels were observed in the ARP/PDRN group compared with the ARP group at coronal, middle, apical and total position at 2-week healing and at coronal, apical and total position at 4-week healing (Figure 4 and Table S3). However, no significant differences were found between the ARP and ARP/PDRN groups at 12 weeks.

3.4 | Three-dimensional volumetric analysis

The results of the volumetric measurements are shown in Figure 5 and Table S4. The mean volumetric changes on the buccal side were −20.12 ± 6.67 mm^3 versus −15.80 ± 3.92 mm^3 (T4-T0, $p = .0078$), −24.14 ± 6.67 mm^3 versus −20.06 ± 4.04 mm^3 (T8-T0, $p = .0234$) and −26.48 ± 5.77 mm^3 versus −21.16 ± 4.66 mm^3

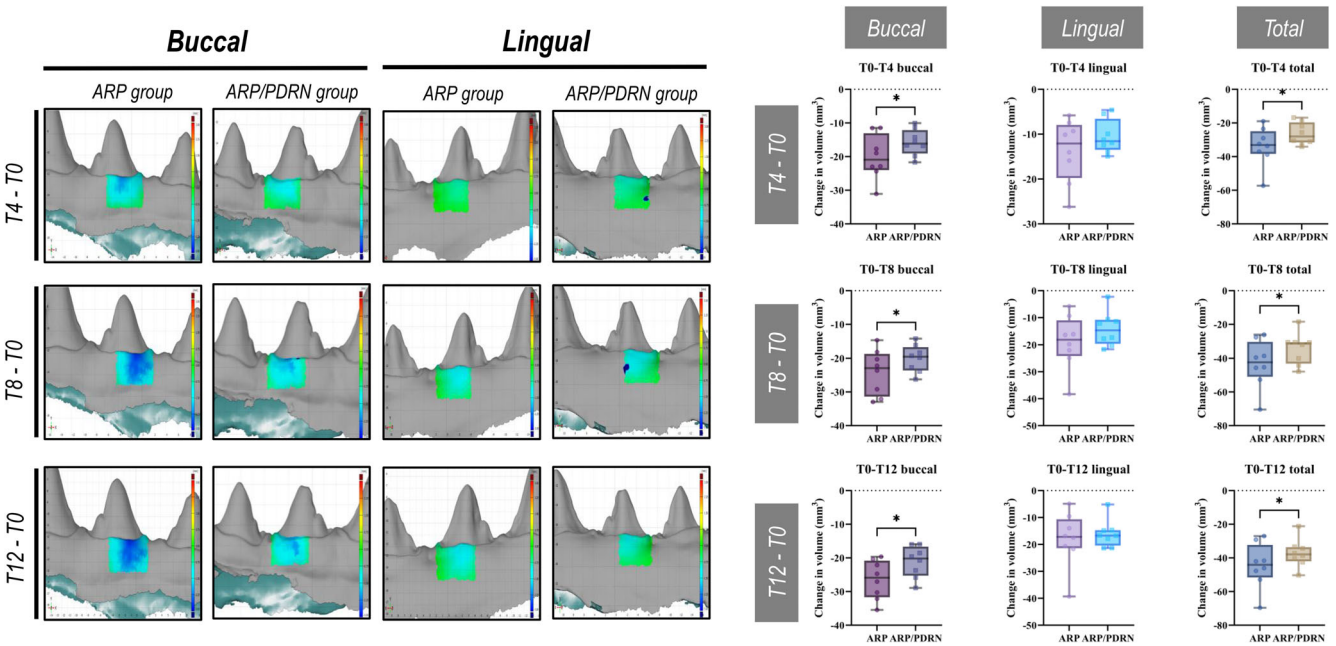

FIGURE 5 Results of three-dimensional volumetric changes in buccal and lingual sides over time. Data are presented with box and whisker plot. T0, 0 weeks; T4, 4 weeks; T8, 8 weeks; T12, 12 weeks of healing. *$p < .05$.

(T12-T0, $p = .0078$) (ARP vs. ARP/PDRN group, respectively). Volume changes on the buccal side were statistically significant between the ARP and ARP/PDRN groups at each time comparison. In contrast, the mean alterations on the lingual side were $-13.74 \pm 7.06\ mm^3$ versus $-10.53 \pm 3.73\ mm^3$ (T4-T0), $-19.08 \pm 10.01\ mm^3$ versus $-14.17 \pm 6.33\ mm^3$ (T8-T0) and $-18.05 \pm 10.25\ mm^3$ versus $-16.07 \pm 5.10\ mm^3$ (T12-T0) (ARP vs. ARP/PDRN group, respectively). No significant differences were observed at any time point. Totally, the ARP/PDRN group exhibited significantly less alveolar bone reduction than the ARP group ($-33.85 \pm 11.57\ mm^3$ vs. $-26.33 \pm 6.49\ mm^3$ for T4-T0 [$p = .0156$], $-43.21 \pm 14.23\ mm^3$ vs. $-34.24 \pm 9.39\ mm^3$ for T8-T0 [$p = .0156$] and $-44.53 \pm 13.47\ mm^3$ vs. $-37.23 \pm 8.38\ mm^3$ for T12-T0 [$p = .0391$]; ARP vs. ARP/PDRN group, respectively).

4 | DISCUSSION

There have been ongoing efforts to identify therapeutic molecules involved in the wound healing and bone regeneration processes. As a regenerative agent, PDRN has been extensively utilized in various medical fields, including diabetic foot ulcers, thermal injuries, rheumatoid arthritis and skin cosmetics. However, despite its cell proliferation, angiogenesis and anti-inflammatory properties, the application of PDRN in dentistry remains relatively unknown. In this context, this study has been conducted to investigate an adjunctive effect of PDRN on bone formation in ARP procedure.

In micro-CT results of this study, improved bone regenerative capacity of PDRN was confirmed in 2-week healing subgroup. One study, about the application of PDRN with block-type alloplastic scaffold (60% hydroxyapatite + 40% β-tricalcium phosphate) in a rabbit calvaria defect model, also reported that radiologically enhanced new bone formation was observed in groups treated with 5 and 10 mg/mL concentrations of PDRN, and the bone regenerative effect of PDRN was comparable to that of recombinant human bone morphogenic protein 2 (rhBMP-2) (Lim et al., 2021). The positive results of PDRN in bone regeneration of a previous study are in accordance with our micro-CT outcomes, but there is a discrepancy in effective therapeutic concentration of PDRN between the two studies. The administration of 1.875 mg/mL of PDRN has shown significantly increased bone formation in this study, while Lim et al. (2021) reported that there were no significant differences under 5 mg/mL concentration of PDRN (0.1 and 1 mg/mL of PDRN group). However, because there are few studies on the application of PDRN in bone regeneration, a direct comparison should be approached with caution. The therapeutic concentration of PDRN in bone regeneration remains unknown, and this concentration may differ when applied to humans. Additionally, the drug delivery system can also impact the outcomes. In this study, because the alloplastic graft material was simply soaked in PDRN solution for 10 min, it was not possible to determine whether PDRN maintained a therapeutic concentration throughout the 12-week healing period. Therefore, these factors are considered as limitations of this study. Further research is needed to identify the therapeutic concentration and effective administration methods of PDRN in bone regeneration treatment.

The histomorphometric measurements in this study revealed that PDRN could promote new bone formation in both the early phases of the bone healing process. Guizzardi et al. (2003, 2007) reported that PDRN enhanced the proliferation of osteoblasts and increased their alkaline phosphatase activity through the A_2 purinergic receptor, ultimately accelerating bone healing in a rat tibia defect model. Our previous study, which focused on maxillary sinus augmentation in a beagle dog model, also confirmed that PDRN improved new bone formation and bone-to-implant contact in the apical augmented area. However, there were no significant differences between the PDRN and control groups in the middle and coronal areas of the augmented sinus (D. Lee et al., 2022). In terms of early new bone formation, these findings are consistent with our observations.

Meanwhile, Aimetti et al. (2009) and Perelman-Karmon et al. (2012) demonstrated that new bone formation in ARP sockets was influenced by the position, with greater new bone formation observed in the apical position compared with the coronal position. M. G. Araujo and Lindhe (2009) also reported that immature bone was observed at the coronal position of ARP sockets using collagenized bovine bone grafts, even after 6 months in a dog experiment. It is thought that the surrounding bone tissue and relatively rich bone-forming conditions of the apical position, compared with the coronal position, may contribute to greater new bone formation in ARP sockets. In contrast, distinct differences between the coronal, middle and apical positions of ARP sockets were not observed in this study. However, significant differences between the ARP and ARP/PRDN groups were found in 2-week healing subgroup. It may be considered that the angiogenic effect of PDRN accelerated new bone formation in the early phase of bone healing. However, in the late phase of bone healing, the osteogenic potential of PDRN is thought to be relatively faded. Therefore, it is believed that PDRN may have clinical advantages to promoting early new bone formation in ARP treatment.

Enhanced angiogenesis is a key feature of PDRN in the tissue regeneration process. Galeano et al. (2008) demonstrated that PDRN stimulated angiogenesis and wound healing in diabetic mice, with increased expression of vascular endothelial growth factor (VEGF), CD31, angioprotein-1 and transglutaminase-II. Moreover, angiogenesis is essential for the bone repair process, as it supplies nutrients, oxygen, growth factors and osteogenic cells (Saran et al., 2014). From this standpoint, the enhancement of newly formed blood vessels driven by PDRN was anticipated to be a critical factor for bone regeneration in this study. An increased number of newly formed blood vessels were observed in ARP/PDRN group compared with ARP group at 2 and 4 weeks of healing. The increased angiogenesis by the PDRN at early phase of wound healing seems to affect the newly formed bone at early phase.

Several previous studies have shown that the dimensional alteration of alveolar bone after tooth extraction is more pronounced on the buccal side due to reduced blood supply and the replacement of bundle bone with woven bone (M. G. Araujo & Lindhe, 2005; Pietrokovski & Massler, 1967; Schropp et al., 2003). Botticelli et al.

(2004) also reported that horizontal resorption of buccal bone was 56%, while the reduction of lingual/palatal bone was 30% during 4 months of healing after extraction (Botticelli et al., 2004). Therefore, ridge preservation may be important in mitigating the dimensional changes resulting from buccal bone resorption. Nevins et al. (2006) documented that buccal bone resorption in ARP sockets with deproteinized bovine bone mineral (DBBM) was less than 20%, but more than 20% in the spontaneous healing group. J. J. Kim, Schwarz, et al. (2017) and J. J. Kim, Ben Amara, et al. (2017) reported that the vertical distance between buccal and lingual crest was significantly reduced in ARP sockets using collagenized DBBM with collagen membrane (1.80 ± 0.16 mm in ARP group and 2.22 ± 0.26 mm in no-graft group) by compensating for buccal bone resorption following tooth extraction. These findings are consistent with our results from three-dimensional volumetric analysis. Additionally, in this study, these protective effects of ARP from ridge volume reductions were synergized with the application of PDRN, especially on the buccal side. PDRN is known to promote cell proliferation like fibroblasts, osteoblasts, endothelial cells and enhance the angiogenesis in the wound repair process (Galeano et al., 2008; Guizzardi et al., 2003; Sini et al., 1999; Thellung et al., 1999). Therefore, it is believed that these regenerative properties of PDRN contribute to the alleviation of ridge volume reduction, and may be particularly significant on the buccal side, where ridge volume alterations are prominent due to the loss of blood supply after tooth extraction.

There are some limitations in this study. In this study, ARP was performed with well-contained bone morphology. Favourable conditions for bone healing may have diminished the significance of the PDRN when evaluating the influence of PDRN on new bone formation. Further experiments using more challenging bone defect models are necessary to elucidate the angiogenic and osteogenic effect of PDRN in the future. In addition, blood vessels were measured without immunohistochemical staining using a von Willebrand factor or CD31 for detecting endothelial cells. For more accurate quantitative analysis of angiogenesis, it is necessary to utilize these markers in the future study.

5 | CONCLUSIONS

Within the limitations of this study, the observations herein suggest that utilizing PDRN as an adjunctive therapeutic agent in ARP may promote new bone formation during the bone healing process and significantly attenuate ridge volume reduction on the buccal side.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to this study. Young-Chang Ko conducted the experiment, analysed the data and wrote the draft of the manuscript. Jungwon Lee conceived the whole experiment design, conducted the statistical analysis and critically revised the manuscript. Istvan Urban interpreted and analysed the data, and critically revised the manuscript. Yang-Jo Seol and Yong-Moo Lee interpreted the data and reviewed the manuscript. Ki-Tae Koo conceived the entire experiment design and critically revised the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Jungwon Lee https://orcid.org/0000-0002-5508-442X
Ki-Tae Koo https://orcid.org/0000-0002-9809-2630

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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