Alveolar ridge preservation using collagenated bone substitutes in two-wall-damaged extraction sockets: an *in vivo* experimental study

Seungho Jeong

The Graduate School
Yonsei University
Department of Dentistry

Alveolar ridge preservation using collagenated bone substitutes in two-wall-damaged extraction sockets: an *in vivo* experimental study

Directed by Professor Jung-Seok Lee

A Dissertation Submitted
to the Department of Dentistry
and the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy in Dental Science

Seungho Jeong

June 2024

This certifies that the Doctoral Dissertation

Of Seungho Jeong is approved.

Thesis Supervisor: Jung-Seok Lee

Ui-Won Jung

Jae-Kook Cha

Jeong-Won Paik

Jin-Young Park

The Graduate School

Yonsei University

June 2024

감사의 글

"나는 단지 해변가에서 조개껍질을 주워 모으며 놀고 있는 아이에 불과하다. 한없이 넓고 깊은 진리의 바다는 여전히 나의 눈앞에 펼쳐져 있다."

- 아이작 뉴턴

박사 학위 논문을 완성하기까지 많은 분들의 도움이 있었습니다. 이 자리를 빌려 저를 지도해 주시고 지지해 주신 모든 분께 진심으로 감사의 말씀을 전합니다.

먼저, 이중석 교수님께 깊은 감사의 말씀을 드립니다. 교수님의 지칠 줄 모르는 열정과 섬세한 지도가 없었다면 이 논문은 완성될 수 없었을 것입니다. 진리에 대한 자세와 연구자로서 나아갈 방향을 제시해 주시고, 언제나 따뜻한 조언과 격려를 아끼지 않으신 교수님의 가르침은 저에게 큰 영감이 되었습니다. 또한, 치주과 전문의로서 임상적으로 갖춰야 할 모든 것들을 채워주시고 끊임없이 성장할 수 있도록 이끌어 주신 점에 대해 깊이 감사드립니다.

정의원, 차재국, 백정원 그리고 박진영 교수님께도 감사의 말씀을 드립니다. 바쁘신 와중에도 제 논문을 심사해 주시고, 소중한 조언과 피드백을 주신 덕분에 논문의 완성도를 높일 수 있었습니다. 심사위원 교수님들께서 주신 귀중한 조언들은 제 연구의 방향성을 바로잡는 데 큰 도움이 되었습니다.

연세대학교 치주과에서 3년간 동고동락한 의국 동기 및 선후배께도 감사의 인사를 전합니다. 공부하고 생활하며 함께 보낸 시간과 경험들은 저에게 큰 힘이 되었습니다. 함께 어려움을 겪으며 나눈 시간이 있었기에, 이 여정을 끝까지 마무리할 수 있었습니다. 여러분과 나눈 우정과 추억은 저의 큰 자산입니다.

가족들에게도 깊은 감사를 드립니다. 항상 변함없는 사랑과 지지로 저를 응원해 주신 부모님, 그리고 모든 순간 곁에서 힘이 되어준 누나와 매형, 귀여운 조카(서은, 서희)의 사랑과 믿음이 있어 이 모든 것을 이뤄낼 수 있었습니다. 특히 어머니께서 보여주신 무한한 사랑과 헌신은 저에게 큰 힘이 되었고, 이 논문을 완성하는 데 있어 원동력이 되었습니다. 이 논문을 여러분께 바칩니다.

마지막으로, 이 연구를 수행할 수 있도록 도와주신 모든 분께 감사의 말씀을 드립니다. 이 논문이 학문과 사회에 작은 기여라도 할 수 있기를 바랍니다. 앞으로도 배움의 자세를 잃지 않고 항상 새로운 진리를 탐구하는 연구자이자 치과의사가 될 것을 다짐합니다.

"무엇을 알든지, 나는 오직 진리를 찾기 위해 노력할 뿐이다."

- 아이작 뉴턴

이제 새로운 시작을 향해 나아가며, 이 자리에 머물지 않고 더 나아갈 것을 다짐합니다.

2024년 여름

정 승 호

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Alveolar ridge preservation using collagenated bone substitutes in two-wall-damaged extraction sockets: an *in vivo* experimental study

Seungho Jeong, B.S., D.M.D.

Department of Dentistry
The Graduate School, Yonsei University

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

Aim: To determine healing outcomes following alveolar ridge preservation (ARP) with two types of collagenated bone substitutes (cross-linked [CL-CB] and non-cross-linked [NCL-CB]) in two-wall-damaged extraction sockets in dogs.

Materials and Methods: Two-wall defects were surgically created in seven beagle dogs and treated in three experimental groups: (i) no grafting (control group), (ii) socket filled with deproteinized bovine bone material and a non-cross-linked collagen matrix (NCL-CB group), and (iii) socket filled with deproteinized porcine bone material and a cross-linked collagen matrix (CL-CB group). One animal was sacrificed after 1 week of healing for tissue assessments using the quantitative reverse-transcription polymerase chain reaction, and the other six were sacrificed after 8 weeks of healing for microcomputed tomography and histological analyses.

Results: The alveolar ridge dimensions showed comparable reductions after tooth extraction across both experimental groups (NCL-CB and CL-CB) and the control group. Qualitative histological analyses revealed that significantly less mineralized tissue formed in both the NCL-CB and CL-CB groups than in the control group ($p < 0.05$). There were

no significant differences in the histological and radiographic results between the two types of collagenated bone substitutes groups (NCL-CB and CL-CB). Compared with the control group, both collagenated bone substitutes groups exhibited moderate increases in the expression levels of vascular endothelial growth factor and fibroblast growth factor-2.

Conclusion: ARP using either cross-linked or non-cross-linked collagenated bone substitute might not be particularly effective in preserving the alveolar ridge dimensions and reducing mineralized tissue formation in two-wall-damaged extraction sockets.

Key words: Alveolar ridge augmentation, Animal model, Bone substitutes, Tooth extraction

1. INTRODUCTION

The alveolar ridge preservation (ARP) technique employs a volume-stable (osteoconductive) xenogeneic bone substitute and is supported by a body of scientific evidence derived from a series of preclinical experiments conducted in dogs (Araújo et al., 2009; Araújo et al., 2008; Araújo et al., 2010; Araújo and Lindhe, 2005, 2009; Araújo et al., 2006; Cardaropoli et al., 2005; Lindhe et al., 2014). These studies have shown that tooth extraction results in dimensional shrinkage of the alveolar ridge, which is particularly pronounced in the buccal region. Moreover, they have demonstrated that compensatory reduction of this dimensional change can be achieved by applying grafting materials. One prominent material utilized in ARP is a collagenated variant of the xenogeneic bone substitute, for which there is now also robust support from various preclinical investigations. Collagenated bone substitutes offer distinct advantages for ARP due to their ease of manipulation and inherent stability, which is attributable to the collagen binder providing a matrix for effectively stabilizing particulate materials in the grafted region. Numerous clinical trials have further substantiated the success of ARP through the use of collagenated bone substitutes (Barone et al., 2008; Barone et al., 2015; Barone et al., 2013; Barone et al., 2016; Gholami et al., 2012; Jung et al., 2013; Mardas et al., 2010; Schneider et al., 2014). Consequently, these materials are now widely considered the preferred choice in the ARP technique.

However, the existing body of scientific evidence does not uniformly support the application of extraction-socket grafting procedures in all clinical scenarios. Initial studies focusing on ARP primarily utilized a single-tooth gap and intact extraction-socket model (Araújo et al., 2009; Araújo et al., 2010; Araújo and Lindhe, 2009). Applying collagenated bone substitutes within such intact extraction sockets notably reduced the resorption of the cross-sectional area in the coronal region of the alveolar process, with values of 3% compared with 25% for grafted versus non-grafted sites, respectively (Araújo et al., 2015). However, dental practitioners in real-world clinical settings frequently encounter extraction sockets with various defects, commonly referred to as damaged extraction sockets. Our

research group has conducted several preclinical experiments on extraction-socket models in which one or two walls are damaged, which revealed that ARP with particulate forms of xenogeneic bone substitutes maintains the original dimensions of the alveolar ridge in both types of damage(Lee et al., 2018b; Lee et al., 2015b; Tien et al., 2021). One important question arising from the findings in both intact and damaged sockets is whether collagenated bone substitutes are suitable for ARP in damaged extraction sockets where the bony walls have been compromised to the extent that they can no longer provide structural support. It is noteworthy that the collagen binder within the material may undergo rapid resorption during the initial healing period, and its occupying volume could potentially collapse in unfavourable defect types, such as those seen in damaged extraction sockets.

Numerous approaches have been applied in attempts to mitigate collagen resorption and extend its ability to support space maintenance, both in collagen membranes and matrices. A prominent approach involves the cross-linking of collagen fibres, which can also be applied to collagen binders within collagenated bone substitutes. Our recent investigation found that soft-type block bone substitutes conjugated with cross-linked collagen exhibited significantly slower resorption than their non-cross-linked counterparts in both *in vitro* and *in vivo* conditions. Notably, the former also enhance space augmentation and new bone formation in a rabbit calvarial augmentation model (study currently under review).

Based on the above background, the primary objectives of the present study were twofold: (i) to comprehensively characterize dimensional alterations and histological bone formation within the alveolar ridge subsequent to the grafting of collagenated bone substitutes into two-wall-damaged extraction sockets, and (ii) to compare the efficacies of two distinct types of collagenated bone substitutes, specifically those employing a non-cross-linked collagen matrix and a cross-linked collagen matrix.

2. MATERIALS AND METHODS

2.1. Animals and materials

Seven beagle dogs weighing around 15 kg and aged 15–24 months were the subjects of this study. Each dog received a uniform diet of soft feed and was housed separately under controlled humidity and temperature. Six of the animals were utilized for histological and radiographic evaluations, while the seventh was used to examine gene expression patterns during the initial healing period. To adhere to the Three R's approach to animal research—Replacement, Reduction and Refinement—the required sample size was determined based on previous studies (Araújo and Lindhe, 2009; Lee et al., 2018b; Park et al., 2023; Tien et al., 2021). The experimental design was approved by the Institutional Animal Care and Use Committee of Yonsei Medical Center in Seoul, South Korea (Approval No. 2021-0316), and was conducted in accordance with the ARRIVE guideline (Percie du Sert et al., 2020).

For the purpose of ARP, two types of commercially available collagenated bone substitutes were employed: the first was designated as NCL-CB, 90% of which comprised particles of deproteinized bovine bone mineral (DBBM) with a size range of 0.25–1.0 mm conjugated with a non-cross-linked porcine collagen matrix as the remaining 10% (Bio-Oss Collagen, Geistlich Pharma AG, Wolhusen, Switzerland); and the second was designated as CL-CB, 85% of which comprised particles of deproteinized porcine bone mineral (DPBM) of size 0.25–1.0 mm conjugated with a cross-linked porcine collagen matrix as the remaining 15% (THE Graf Collagen, Purgo Biologics, Seongnam, South Korea).

2.2. Study design

This study investigated a two-wall-damaged extraction-socket model that we have reported on previously (Park et al., 2023; Tien et al., 2021). Following tooth hemisection, distal roots of the unilateral mandibular second, third and fourth premolars were extracted, and the entire buccal and lingual bone walls were removed as illustrated in Figure 1B. Mesial roots were left intact, serving both as the pristine control sites and as baselines for the measurements of dimensional changes. These measurements were later compared with those made at the distal grafted sites. Three types of damaged extraction sockets were categorized into the following groups:

1. Control group: Sockets underwent spontaneous healing with no grafting;
2. NCL-CB group: Sockets were filled with NCL-CB;
3. CL-CB group: Sockets were filled with CL-CB.

Group allocation for each premolar site in the first dog was determined through randomization, while groups were rotationally allocated for the remaining dogs across three experimental sites. This approach ensured that each group was evenly distributed among the three premolar sites.

2.3. Surgical protocols

General anaesthesia was induced using medetomidine (0.75 mg/kg, intramuscularly; Tomidin, Provet Veterinary Products, Istanbul, Turkey) and alfaxalone (2 mg/kg, intravenously; Jurox, Rutherford, NSW, Australia), and maintained via isoflurane inhalation (Forane, Choongwae Pharmaceutical, Seoul, South Korea). Following the administration of local anaesthesia with 2% lidocaine hydrochloride (dilution of 1:80,000; Kwangmyung Pharm, Seoul, South Korea), a full-thickness flap was elevated at the

experimental site in the distal-root region only.

Subsequent to tooth hemisectioning and defect creation, designated biomaterials were applied to the basal bone width according to the group allocation: control, NCL-CB or CL-CB. Overaugmentation was conducted up to the crestal area in accordance with the predetermined experimental group allocation (Figure 1A and B). The flap was then carefully repositioned and secured with 6-0 monofilament sutures (Monosyn, B. Braun Medical, Bethlehem, PA, USA) to achieve primary closure.

Post-operative care included administering an analgesic (0.2 mg/kg; meloxicam, Boehringer Ingelheim, Ingelheim, Germany) and an antibiotic (20 mg/kg; cefazolin sodium, Yuhan, Seoul, South Korea) once daily for 7 days until suture removal. The animals were closely monitored by veterinarians throughout the healing process. Weekly checks of the surgical sites were performed under sedation to identify any anomalies, with intravenous sedation administered as necessary. Oral prophylaxis was delivered concurrently to the animals. The animals were euthanized after an 8-week healing period before performing the radiographic and histological analyses.

2.4. Microcomputed tomography radiographic analysis

The obtained samples were fixed in a 10% neutral buffered formalin solution for 2 weeks before performing microcomputed tomography (micro-CT) scanning (SkyScan 1173, SkyScan, Aartselaar, Belgium) with the following parameters: field of view = 6.2 cm, projection times = 40 min and 41 s, number of projections = 799 and frame averaging = 4. The resolution achieved during the scans was 35 μm under conditions of 130 kV and 60 μA. Scanning data in DICOM format were subsequently transferred to computer software (OnDemand3D, Cybermed, Seoul, South Korea) for three-dimensional reconstruction.

Based on the assumption of minimal differences in the original ridge dimensions between the mesial and distal sites of each tooth, the ridge dimensions surrounding the

intact mesial root served as the pre-extraction baseline for measuring post-operative dimensional changes at the distal grafted site. Cross-sectional images of the mesial root (serving as baseline) and the centre of the grafted site (after 8 weeks) were superimposed using the mandibular canal and the border of the mandible as reference points (Figure 2A). The ridge crest and dental apex were employed to designate the areas of interest in the coronal and apical regions, respectively.

Standard software (Adobe Photoshop CC 2021, Adobe Systems, San Jose, CA, USA) was used to calculate the proportional changes in the ridge area between baseline and after 8 weeks of healing.

2.5. Histological analysis

After performing a thorough decalcification process, each unilateral mandible was meticulously sectioned into six blocks incorporating three mesial pristine sites and three corresponding grafted sites. After decalcification, the samples were sequentially dehydrated using graded ethanol solutions before being trimmed and then embedded in paraffin. The most-central buccolingual section was cut along the dental root axis or the extraction socket at a thickness of 4 μm and then stained with haematoxylin, eosin and Masson's trichrome for the preparation of histological slides (Figures 1C and 2B). Digital slides were scanned at 200× magnification (PANNORAMIC 250 Flash III, 3DHISTECH, Budapest, Hungary). Histomorphometric data were extracted from the digitally scanned images using standard software (Adobe Photoshop CC 2021, Adobe Systems).

Following verification that a histological section corresponded precisely with the midsagittal plane of the experimental area in micro-CT, histological images from mesial pristine sites and distal experimental sites were superimposed using the mandibular canal and the border of the mandible as reference points (Figure 2B). The following planimetric measurements were subsequently made:

- Augmented ridge area (ARA): defined by the outermost margin of the grafted site.
- Regenerated ridge area (RRA): defined by the outermost margin of the newly formed bone.
- Residual biomaterials: the areas occupied by the residual DBBM or DPBM particles relative to the ARA.
- Mineralized tissue: the area of newly formed bone relative to the ARA.
- Fibrovascular tissue: the area occupied by non-mineralized tissue relative to the ARA.

2.6. Quantitative reverse-transcription polymerase chain reaction

The mRNA expression levels of target genes, which included those 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, interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), were assessed using the quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Briefly, total RNA was extracted from tissue samples using TRIzol (Invitrogen, Frederick, MD, USA). cDNA was then synthesized from the isolated RNA using oligo (dT) primers. The qRT-PCR procedure was performed with SYBR Premix Ex Taq II (Tli RNase H Plus) reagents (RR82LR, Takara, Ann Arbor, MI, USA) on a device from Applied Biosystems (Foster City, CA, USA). The cycling protocol for the qRT-PCR comprised an initial denaturation stage at 95°C for 10 minutes, followed by 45 cycles of 15 seconds of denaturation at 95°C, 15 seconds of annealing and 15 seconds of extension at 72°C. The expression levels of the target genes were normalized to that for GAPDH, and their relative quantities were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.7. Statistical analyses

Statistical analyses were conducted using SPSS software (version 26, SPSS, Chicago, IL, USA). Parameters were presented as mean±standard deviation values. All data were consistent with the assumption of sphericity, and the normality of the data distribution was assessed using the Kolmogorov–Smirnov test. The primary outcome variable of the study was the ARA, and the unit of analysis was the individual specimen. Analysis of variance was used for comparisons between the experimental groups. In all analyses, statistical significance was considered to be present when $p < 0.05$ in a two-tailed test.

3. RESULTS

3.1. Clinical findings

All experimental sites showed uneventful healing without any inflammation or wound dehiscence during the 8-week healing period. Horizontal and vertical collapse of the alveolar ridge dimensions appeared at all of the experimental sites without grafting compared with those at the adjacent reference sites in the residual mesial-root region.

3.2. Radiographic observations

When compared with the baseline, all experimental groups showed dimensional shrinkage of the alveolar ridge after 8 weeks of healing (Figure 3). The grafted materials were observed inside the extraction sockets in the two intervention groups (NCL-CB and CL-CB). Due to the residual biomaterials, the grafted areas showed higher radiopacity than at the baseline. However, in the superficial area of the graft, the grafted bone substitutes appeared to be loosely oriented.

3.3. Quantitative analysis of superimposed micro-CT image

The ridge dimensions had decreased to 55.41±12.95%, 83.29±24.96% and 73.46±16.59% of the baseline values in the control, NCL-CB and CL-CB groups, respectively (Table 1, Figure 4), with no significant differences between the groups ($p > 0.05$).

3.4. Histological observations

There was no pronounced new bone formation within the sockets in any of the experimental groups. The regenerated alveolar ridges could be clearly distinguished from the superficial loose connective tissues by a border of new bone (Figure 5). More new bone was formed in the apical regions of the socket than in the coronal regions, and this bone also appeared to be more mature. There were no significant differences in the bone formation patterns between the NCL-CB and CL-CB groups, even in the outermost regions.

3.5. Quantitative and qualitative analyses of superimposed histological image

The ARAs had decreased to 68.75±14.20%, 79.88±20.05% and 76.10±21.09% of the baseline values in the control, NCL-CB and CL-CB groups, respectively, while the RRAs had decreased to 68.75±14.20%, 38.46±26.50% and 40.93±13.95% (Table 1, Figure 5). The only significant difference was in the RRA between the control and NCL-CB groups ($p = 0.045$).

Qualitative analysis of the ARAs indicated that the proportion of mineralized tissue was significantly higher in the control group than in the other two experimental groups ($p < 0.05$), while the compositions of fibrovascular tissues did not differ significantly between the three groups ($p > 0.05$). The proportions of mineralized tissues (residual biomaterials) were 67.15±14.35% (not measured), 25.28±10.40% (30.96±5.97%) and 29.86±12.04% (21.53±8.87%) in the control, NCL-CB and CL-CB groups, respectively; the corresponding proportions of fibrovascular tissues were 32.85±14.35%, 43.76±7.23% and 48.61±8.41% (Figure 5).

3.6. Expression patterns of genes encoding growth factors

The gene expression patterns were evaluated in one animal after 1 week of healing in order to assess the initial healing process (Figure 6). The expression levels for two of the tested growth factors (VEGF and FGF-2) were upregulated in the NCL-CB and CL-CB groups relative to the control group, by 1.56- and 1.77-fold, and 1.57- and 1.86-fold, respectively. The expression levels for the other tested growth factors (BMP-2 and TGF-ß) in the NCL-CB and CL-CB groups did not differ significantly from those in the control group (changing 0.72- and 0.97-fold, and 1.06- and 0.82-fold, respectively), and no specific trends were observed between the two intervention groups or between these intervention groups and the control group. The expression levels for osteocalcin (an osteoblast-releasing cytokine) and the transmembrane calcitonin receptor (which inhibits osteoclastic activity) in the NCL-CB and CL-CB groups also did not differ from those in the control group (changing 0.87- and 0.92-fold, and 0.81- and 0.50-fold, respectively). Lastly, IL-6 and TNF-α, which are inflammatory cytokines activating osteoclastogenesis, did not show specific trends in the NCL-CB and CL-CB groups relative to the control group (changing 1.20- and 0.86-fold, and 1.01- and 0.79-fold, respectively.

4. DISCUSSION

This study compared dimensional alterations and bone healing following the use of two collagenated bone substitutes (NCL-CB and CL-CB) without a covering membrane in two-wall-damaged extraction sockets. The primary findings were that (i) the two intervention groups (receiving NCL-CB and CL-CB) and the control group exhibited comparable reductions of the alveolar ridge dimensions after tooth extraction, indicating relatively minor effects on alveolar preservation by the collagenated bone substitutes; (ii) both intervention groups showed significantly reductions in mineralized tissue formation compared with the control group; and (iii) there were no additional impacts of using cross-linking technology in the collagenated bone substitute for managing two-wall-damaged extraction sockets.

The present results conflict with previous findings for ARP using NCL-CB (Araújo and Lindhe, 2009). This discrepancy is attributable to differences in the experimental models: the previous study employed intact extraction sockets, whereas our study utilized two-wall-damaged extraction-socket models. However, this also contrasts with our previous finding of successful ARP even in the same (two-wall-damaged) experimental model (Tien et al., 2021); that is, the collagenated bone substitute used in the present study failed to maintain the alveolar ridge dimensions in the compromised defect of a two-wall-damaged extraction socket. This could be due to degradation of the collagen matrix in the early healing phase, which probably leads to the loss of the inherent stability of the grafted materials, subsequently compromising the space maintenance. A systematic review (Avila-Ortiz et al., 2019) found that no method from among various ARP treatment options was superior based on the available evidence. Considering that most previous studies of ARP investigated regions with intact extraction sockets, further studies are necessary to ascertain the optimal ARP treatment modality in damaged extraction sockets.

The use of collagenated bone substitutes in this study had negative effects on the quality of new bone and the formation of mineralized tissue. It might be reasonable to assume that

the quality of new bone in the intervention groups would have been enhanced by the innate ability of collagen to promote wound healing and tissue regeneration by providing a natural scaffold. Notably, the qRT-PCR results emphasized the upregulation of VEGF and FGF-2 in both the NCL-CB and CL-CB groups, which indicated the facilitation of angiogenesis and fibroblast growth. However, the amount of mineralized tissue was reduced in both intervention groups that received bone grafting materials, which is consistent with previous findings for both intact and damaged sockets (Araújo and Lindhe, 2009; Hong et al., 2014; Lee et al., 2018b). Results for the sequential healing after grafting various materials in extraction sockets indicate that the initial tissue responses included immense infiltration of inflammatory cells before the following processes of osteoclast/osteoblast induction (Hong et al., 2014). Considering the previous results showing comparable mineralized tissue formation (30.35%) at sites that received bone substitute only (Lee et al., 2018b), there appears to be no correlation between collagen matrix conjugation in xenogeneic bone materials and bone regeneration or remodeling.

Several previous studies investigated the efficacy of cross-linked collagen membranes in guided bone regeneration (GBR). Animal studies have demonstrated slower degradation when using a cross-linked collagen matrix (Naenni et al., 2020; Rothamel et al., 2005), and clinical studies (Lee et al., 2015a; Tal et al., 2008) have suggested that a cross-linked matrix (membrane) enhances durability and integrity, making it potentially beneficial in GBR procedures. We therefore originally hypothesized that CL-CB is resorbed less than the cross-linked matrix to provide more space for regeneration. However, no discernible differences were observed between our two intervention models. It was intriguing that our histological evaluations revealed a slight increase in residual biomaterials in the NCL-CB group. In our previous randomized clinical trial (Lee et al., 2018a), two different particle-type xenografts displayed similar augmented outcomes in damaged extraction sockets. Another animal study (Benic et al., 2016) showed that using different types of xenografts (particulate xenograft and two different block xenografts) for GBR also had no significant effect on amount of new bone formation. These findings suggest that other factors, such as mechanical support for stability or bone defect morphology, are more influential than the presence of cross-linking collagen matrix in the bone substitute. It appears that the cross-

linking collagen matrix in the bone substitutes might not crucially affect the outcome of ARP; a well-designed experimental study is imperative to further explore this observation.

This study was subject to some limitations. Firstly, an optimal experimental design would incorporate a positive control group (traditional GBR technique, using particulate bone graft materials and collagen membranes), which was absent in this study due to the small number of premolars (only three) with a similar condition in the unilateral alveolar ridge. Secondly, the collagenated bone substitutes used in this study had different material compositions. This represents a limitation since it is unclear whether the results of the histometric analysis (which showed more residual material in the NCL-CB than the CL-CB group) were attributable to variations in the sizes of residual particles, the presence or absence of cross-linking, or another factor. Within the normal limitations of animal experiments, it seems necessary to interpret the results while acknowledging that they exhibit trends consistent with the main findings. Moreover, our previous study (Lee et al., 2018b) observed the pattern of bone regeneration over time using this analytical approach to assess the regeneration degree associated with using collagenated bone substitutes. Given the relatively short observation period of the present study relative to other animal experimental studies, interpretations should be made conservatively.

5. CONCLUSION

ARP using either cross-linked or non-cross-linked collagenated bone substitute might not be particularly effective at preserving the alveolar ridge dimensions or reducing mineralized tissue formation in two-wall-damaged extraction sockets.

REFERENCES

Araújo M, Linder E, Lindhe J (2009). Effect of a xenograft on early bone formation in extraction sockets: an experimental study in dog. *Clin Oral Implants Res* 20(1): 1-6.

Araújo M, Linder E, Wennström J, Lindhe J (2008). The influence of Bio-Oss Collagen on healing of an extraction socket: an experimental study in the dog. *Int J Periodontics Restorative Dent* 28(2): 123-135.

Araújo MG, da Silva JCC, de Mendonça AF, Lindhe J (2015). Ridge alterations following grafting of fresh extraction sockets in man. A randomized clinical trial. *Clin Oral Implants Res* 26(4): 407-412.

Araújo MG, Liljenberg B, Lindhe J (2010). Dynamics of Bio-Oss Collagen incorporation in fresh extraction wounds: an experimental study in the dog. *Clin Oral Implants Res* 21(1): 55-64.

Araújo MG, Lindhe J (2005). Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *J Clin Periodontol* 32(2): 212-218.

Araújo MG, Lindhe J (2009). Ridge preservation with the use of Bio-Oss collagen: A 6-month study in the dog. *Clin Oral Implants Res* 20(5): 433-440.

Araújo MG, Sukekava F, Wennström JL, Lindhe J (2006). Tissue modeling following implant placement in fresh extraction sockets. *Clin Oral Implants Res* 17(6): 615-624.

Avila-Ortiz G, Chambrone L, Vignoletti F (2019). Effect of alveolar ridge preservation interventions following tooth extraction: A systematic review and meta-analysis. *J Clin Periodontol* 46 Suppl 21: 195-223.

Barone A, Aldini NN, Fini M, Giardino R, Calvo Guirado JL, Covani U (2008). Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorphometric study. *J Periodontol* 79(8): 1370-1377.

Barone A, Borgia V, Covani U, Ricci M, Piattelli A, Iezzi G (2015). Flap versus flapless

procedure for ridge preservation in alveolar extraction sockets: a histological evaluation in a randomized clinical trial. *Clin Oral Implants Res* 26(7): 806-813.

Barone A, Ricci M, Tonelli P, Santini S, Covani U (2013). Tissue changes of extraction sockets in humans: a comparison of spontaneous healing vs. ridge preservation with secondary soft tissue healing. *Clin Oral Implants Res* 24(11): 1231-1237.

Barone A, Toti P, Quaranta A, Alfonsi F, Cucchi A, Calvo-Guirado JL, et al. (2016). Volumetric analysis of remodelling pattern after ridge preservation comparing use of two types of xenografts. A multicentre randomized clinical trial. *Clin Oral Implants Res* 27(11): e105-e115.

Benic GI, Thoma DS, Muñoz F, Sanz Martin I, Jung RE, Hämmerle CH (2016). Guided bone regeneration of peri-implant defects with particulated and block xenogenic bone substitutes. *Clin Oral Implants Res* 27(5): 567-576.

Cardaropoli G, Araújo M, Hayacibara R, Sukekava F, Lindhe J (2005). Healing of extraction sockets and surgically produced - augmented and non-augmented - defects in the alveolar ridge. An experimental study in the dog. *J Clin Periodontol* 32(5): 435-440.

Gholami GA, Najafi B, Mashhadiabbas F, Goetz W, Najafi S (2012). Clinical, histologic and histomorphometric evaluation of socket preservation using a synthetic nanocrystalline hydroxyapatite in comparison with a bovine xenograft: a randomized clinical trial. *Clin Oral Implants Res* 23(10): 1198-1204.

Hong JY, Lee JS, Pang EK, Jung UW, Choi SH, Kim CK (2014). Impact of different synthetic bone fillers on healing of extraction sockets: an experimental study in dogs. *Clin Oral Implants Res* 25(2): e30-37.

Jung RE, Philipp A, Annen BM, Signorelli L, Thoma DS, Hämmerle CH, et al. (2013). Radiographic evaluation of different techniques for ridge preservation after tooth extraction: a randomized controlled clinical trial. *J Clin Periodontol* 40(1): 90-98.

Lee JH, Lee JS, Baek WS, Lim HC, Cha JK, Choi SH, et al. (2015a). Assessment of dehydrothermally cross-linked collagen membrane for guided bone regeneration around

peri-implant dehiscence defects: a randomized single-blinded clinical trial. *J Periodontal Implant Sci* 45(6): 229-237.

Lee JS, Cha JK, Kim CS (2018a). Alveolar ridge regeneration of damaged extraction sockets using deproteinized porcine versus bovine bone minerals: A randomized clinical trial. *Clin Implant Dent Relat Res* 20(5): 729-737.

Lee JS, Choe SH, Cha JK, Seo GY, Kim CS (2018b). Radiographic and histologic observations of sequential healing processes following ridge augmentation after tooth extraction in buccal-bone-deficient extraction sockets in beagle dogs. *J Clin Periodontol* 45(11): 1388-1397.

Lee JS, Jung JS, Im GI, Kim BS, Cho KS, Kim CS (2015b). Ridge regeneration of damaged extraction sockets using rhBMP-2: an experimental study in canine. *J Clin Periodontol* 42(7): 678-687.

Lindhe J, Cecchinato D, Donati M, Tomasi C, Liljenberg B (2014). Ridge preservation with the use of deproteinized bovine bone mineral. *Clin Oral Implants Res* 25(7): 786-790.

Mardas N, Chadha V, Donos N (2010). Alveolar ridge preservation with guided bone regeneration and a synthetic bone substitute or a bovine-derived xenograft: a randomized, controlled clinical trial. *Clin Oral Implants Res* 21(7): 688-698.

Naenni N, Lim HC, Strauss FJ, Jung RE, Hämmerle CHF, Thoma DS (2020). Local tissue effects of various barrier membranes in a rat subcutaneous model. *J Periodontal Implant Sci* 50(5): 327-339.

Park JY, Hong KJ, Ko KA, Cha JK, Gruber R, Lee JS (2023). Platelet-rich fibrin combined with a particulate bone substitute versus guided bone regeneration in the damaged extraction socket: An in vivo study. *J Clin Periodontol* 50(3): 358-367.

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol* 18(7): e3000410.

Rothamel D, Schwarz F, Sager M, Herten M, Sculean A, Becker J (2005). Biodegradation

of differently cross-linked collagen membranes: an experimental study in the rat. *Clin Oral Implants Res* 16(3): 369-378.

Schneider D, Schmidlin PR, Philipp A, Annen BM, Ronay V, Hämmerle CH, et al. (2014). Labial soft tissue volume evaluation of different techniques for ridge preservation after tooth extraction: a randomized controlled clinical trial. *J Clin Periodontol* 41(6): 612-617.

Tal H, Kozlovsky A, Artzi Z, Nemcovsky CE, Moses O (2008). Long-term bio-degradation of cross-linked and non-cross-linked collagen barriers in human guided bone regeneration. *Clin Oral Implants Res* 19(3): 295-302.

Tien HK, Lee WH, Kim CS, Choi SH, Gruber R, Lee JS (2021). Alveolar ridge regeneration in two-wall-damaged extraction sockets of an in vivo experimental model. *Clin Oral Implants Res* 32(8): 971-979.

TABLES

Table 1. Dimensional alterations identified in radiographic and histomorphometric (augmented ridge and regenerated ridge) analyses of the three experimental groups: (i) spontaneous healing group (control) with no grafting, (ii) deproteinized bovine bone mineral conjugated with a non-cross-linked collagen matrix (NCL-CB) and (iii) deproteinized porcine bone mineral conjugated with a cross-linked collagen matrix (CL-CB).

	Control		NCL-CB		CL-CB		*p*
Radiography (augmented ridge)	55.41±12.95	[41.82–69.00%]	83.29±24.96	[57.09–109.49%]	73.46±16.59	[56.05–90.87%]	0.062
Histology (augmented ridge)	68.75±14.20	[53.85–83.65%]	79.88±20.05	[58.85–100.93%]	76.10±21.09	[53.97–98.22%]	0.588
Histology (regenerated ridge)	68.75±14.20	[53.85–83.65%]	38.46±26.50	[10.64–66.27%]	40.93±13.95	[26.30–55.56%]	0.027*

Note: The augmented ridge refers to the entire grafted area, and the regenerated ridge refers to the area containing newly formed bone within the augmented ridge. Data are dimensional percentages (augmented ridge or regenerated ridge) of the area of the alveolar ridge at the experimental site relative to the pre-extraction baseline, presented as mean±standard deviation [95% confidence interval] values.

FIGURES

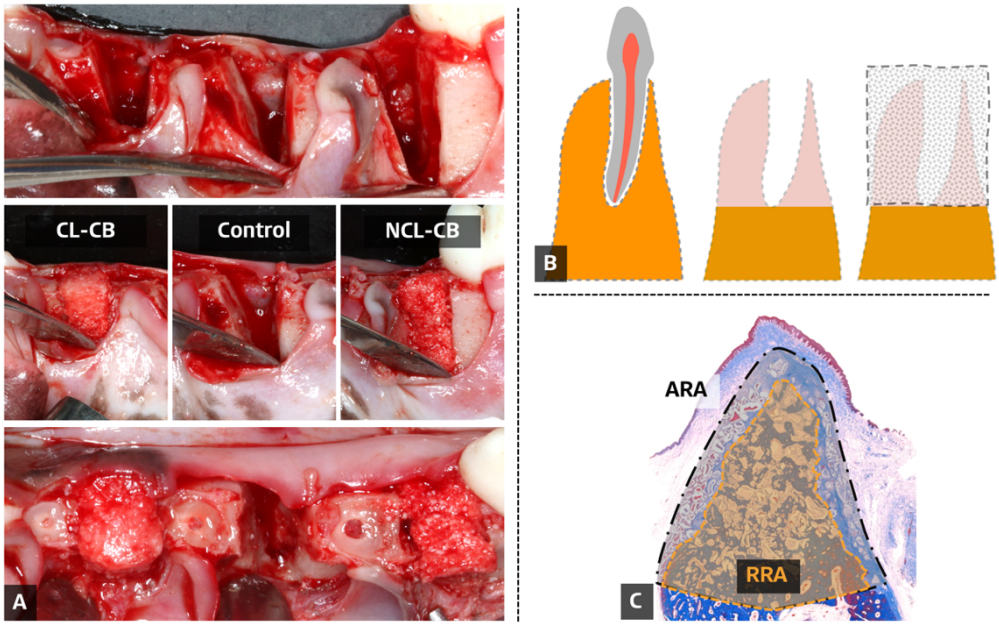

Figure 1. Experimental procedures. (A) After extracting the distal roots of the second, third and fourth mandibular premolars, the buccal and lingual walls were removed (top image). The defects were randomly allocated to three experimental groups: (i) deproteinized porcine bone mineral conjugated with a cross-linked collagen matrix (CL-CB), (ii) spontaneous healing group (control) with no grafting and (iii) deproteinized bovine bone mineral conjugated with a non-cross-linked collagen matrix (NCL-CB) (middle images). The mesial roots were decoronated and preserved as references for the original ridge dimensions (bottom image). (B) All of the buccal and lingual alveolar bones were removed to produce two-wall-damaged extraction sockets in which the mesial and distal walls remained after removing each distal root. The vertical level of the defects was defined based on the pre-existing root apex. After the collagenated bone substitutes were trimmed to fit the width of the basal ridge, overaugmentation was conducted up to the crestal area. (C) The augmented ridge area (ARA) is indicated by the black dotted-dashed line showing the outermost margin of grafted biomaterials. The regenerated ridge area (RRA) is indicated by the red dashed line showing the outermost mineralized area.

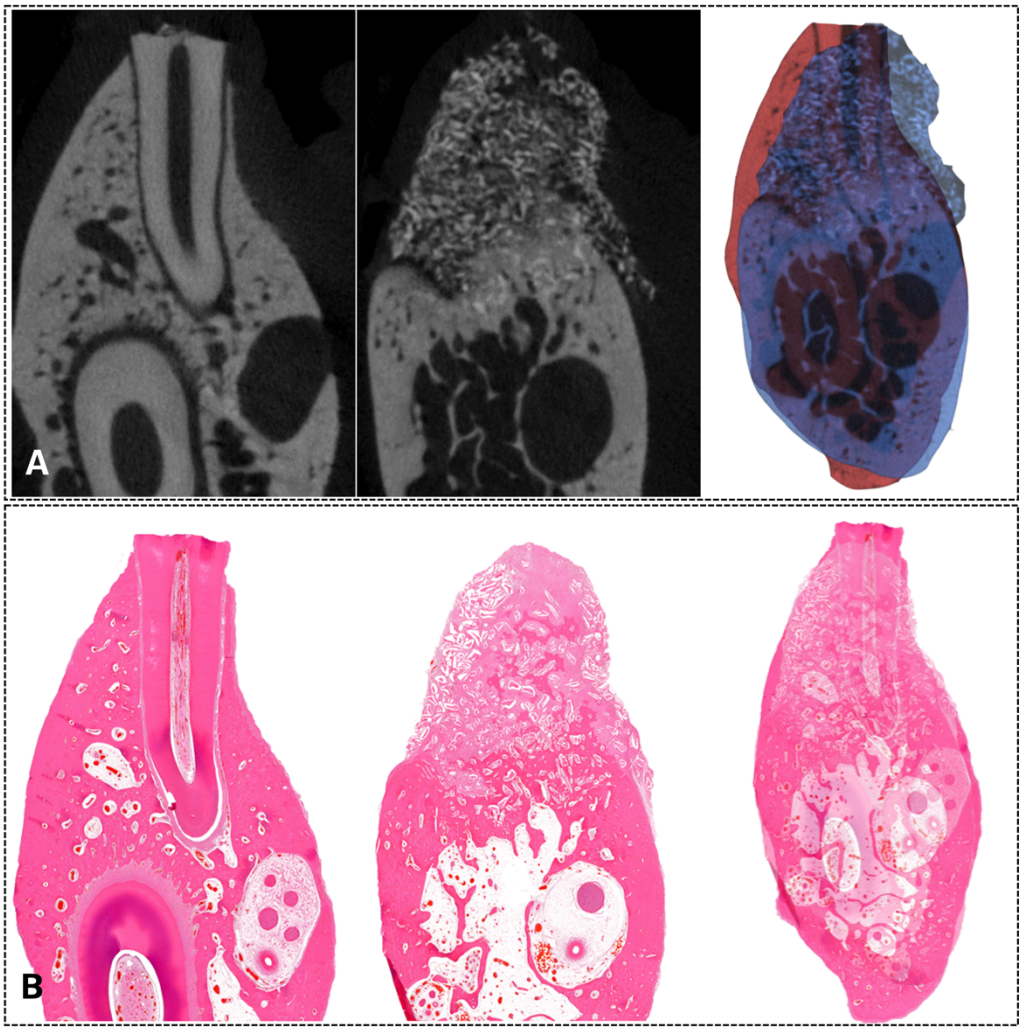

Figure 2. The ridge dimensions around the mesial root were used as the baseline reference (left images) for measuring dimensional changes at the corresponding distal experimental sites (middle images) after 8 weeks of healing. The two sites were superimposed (right images) to measure the percentage changes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

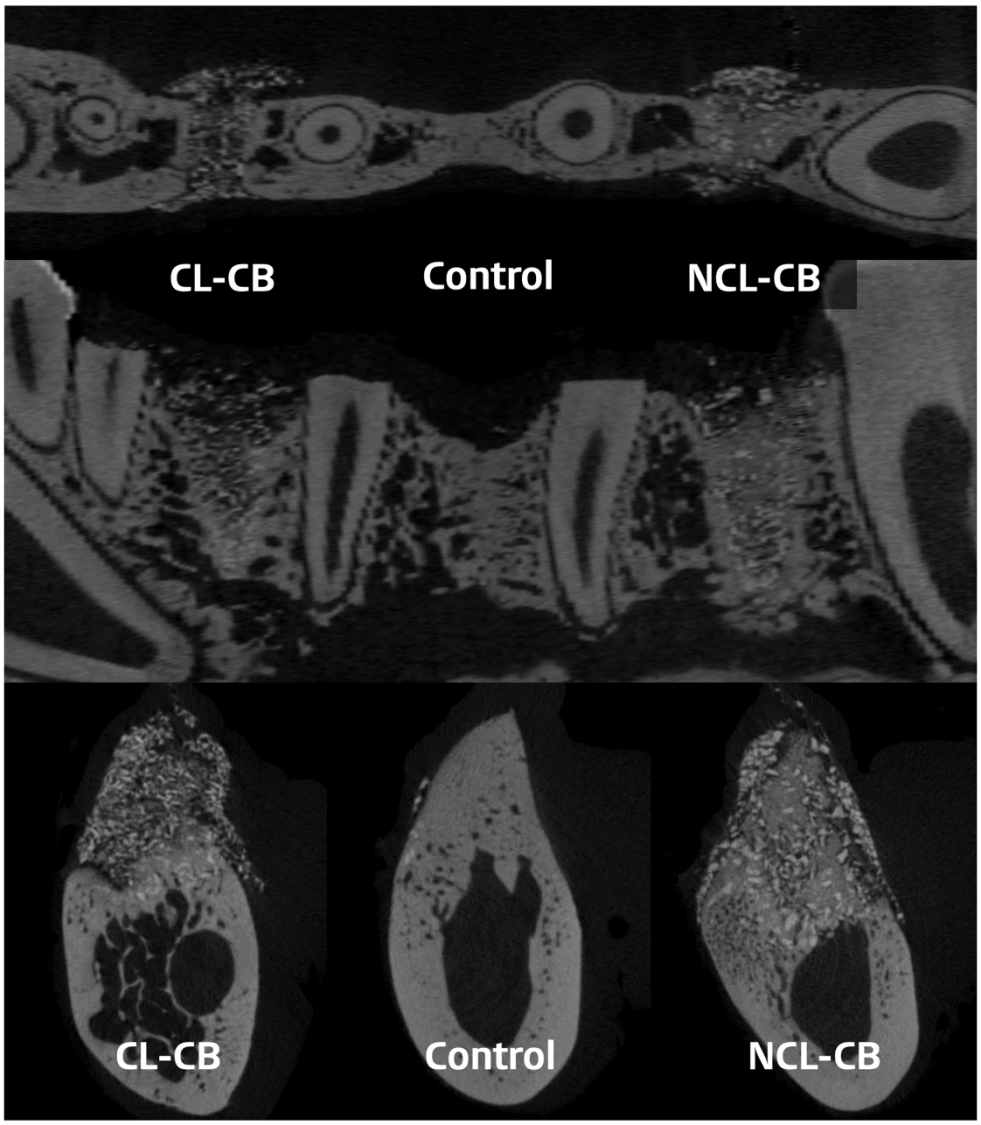

Figure 3. Micro-CT views of the three groups allocated at each experimental site. The top and middle images show axial and panoramic views, respectively, of the three experimental sites and pristine sites of the unilateral mandible. The bottom images show cross-sectional views of the experimental sites. The alveolar ridge dimensions were not preserved at any experimental site relative to the respective pristine site at the mesial root.

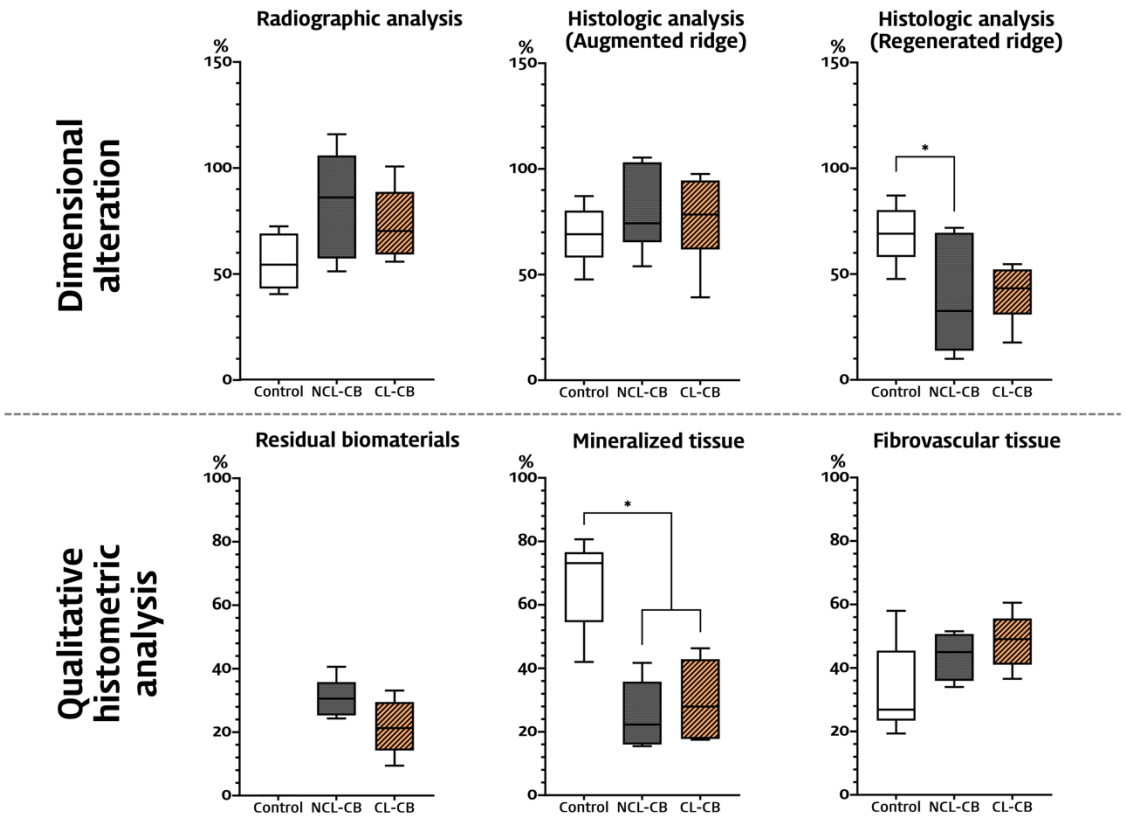

Figure 4. Box plots of dimensional alterations from baseline (pre-extraction) to 8 weeks after alveolar ridge preservation (upper) at the experimental sites and of the results from qualitative histological analyses (lower) of the three experimental groups. The only significant difference in regenerated ridge dimensions was between the control and NCL-CB groups, and the qualitative analysis of mineralized tissue in the control group differed significantly from those in the NCL-CB and CL-CB groups. *$p < 0.05$.

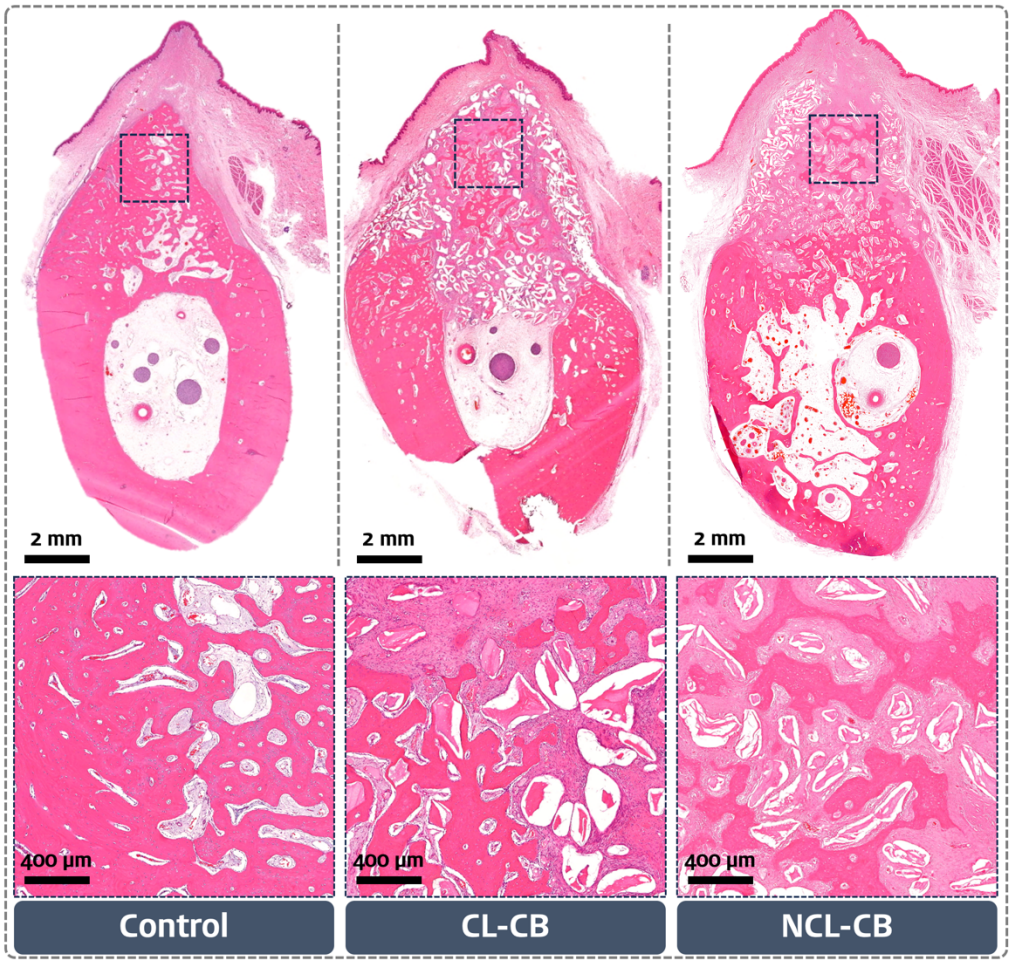

Figure 5. Histological sections of the three experimental groups at high and low magnifications after staining with haematoxylin and eosin. Preservation of alveolar ridge dimensions was not observed in the NCL-CB and CL-CB groups. A reduction in mineralized tissue was evident in the two intervention groups. There was more new bone formation in the apical regions of the socket than in the coronal regions, and this bone was more mature.

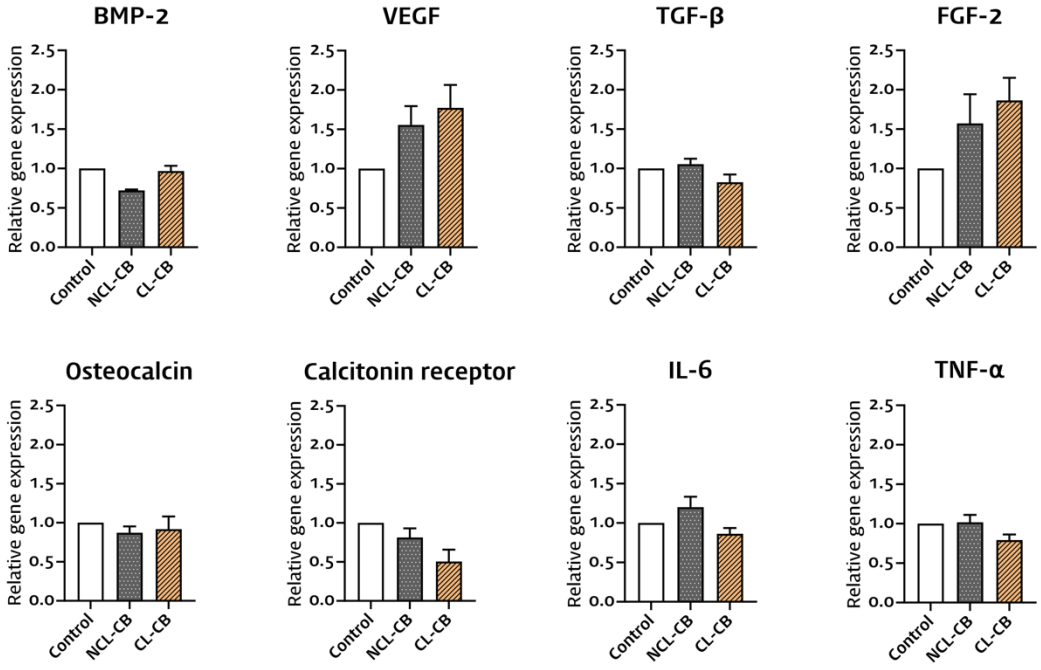

Figure 6. Box plots comparing the gene expression levels of bioactive factors between the three experimental groups after 1 week, as revealed by the quantitative reverse-transcription polymerase chain reaction. Only the gene expression levels of vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) were upregulated in the NCL-CB and CL-CB groups relative to the control group. Data are mean and standard-deviation values. BMP-2, bone morphogenic protein-2; IL-6, interleukin-6; TGF-β, transforming growth factor-β; TNF-α, tumour necrosis factor-α.

Abstract in Korean

2벽성으로 파괴된 발치와 처치 시 콜라겐 함유 골이식재의 치조제 보존 효과에 관한 전임상연구

<지도교수 **이 중 석**>

연세대학교 대학원 치의학과

정 승 호

성견에서의 전임상연구를 통한 과학적 근거를 바탕으로 치조제 보존술은 부피 유지 (골전도성) 이종골 이식재의 도입과 함께 발전되었다. 최근 콜라겐 함유 골이식재는 쉬운 조작성과 재료 본연의 안정성 때문에, 치조제 보존술 뿐만 아니라 다양한 치과 영역에서 사용되고 있다. 콜라겐 함유 골이식재의 치조제 보존술에 대한 효과는 건전한 발치와에서 그 효과가 입증되었다. 하지만 임상 환경에서 만나게 되는 다양한 유형의 파괴된 발치와에 대한 골이식 후 치유 과정에 대한 근거가 제한적이다. 입자형 이종골 이식재는 이전의 여러 파괴된 발치와 모델에서 치조제 보존을 할 수 있다고 평가되었다. 그러나 파괴된 발치와에서의 콜라겐 함유 골 이식재의 치조제 보존에 관한 연구가 제한적이다. 따라서 본 연구에서는 성견에 2벽성으로 파괴된 발치와 모델을 형성하여 두 종류의 콜라겐 함유 골이식재를 이식한 후 치유 결과를 방사선학적 및 조직학적으로 평가하는 것을 목적으로 하였다.

7마리 성견의 하악 편측의 제2, 3, 4 소구치를 편측 절단하고 원심 치근을 발거한 후, 협측 및 설측 골벽을 외과적으로 삭제하여 2벽성 골 결손부를 형성했다. 대조군(Control)에는 어떠한 처치도 하지 않았고, 실험군 1(NCL-CB)에는 비가교성 콜라겐 기질을 혼합한 탈단백 소 유래 골이식재를 이식하였고, 실험군 2(CL-CB)에는 가교성 콜라겐 기질을 혼합한 탈단백 돼지 유래 골이식재를 이식하여 치조제 보존술을 시행하였다. 1주일 후 콜라겐

함유 골이식재의 성장인자 방출 효과를 평가하기 위해 한 마리의 성견을 대상으로 정량적 역전사 중합효소 연쇄 반응을 시켰다. 나머지 6 마리의 성견은 치유 8 주 후에 방사선학적 및 조직학적 분석을 시행했다. 치근을 발거하지 않은 근심측과 치조제 보존술을 시행한 원심측의 방사선 및 조직 영상을 중첩하여 치조제의 면적 변화량을 계측하였다. 실험군과 대조군은 모두 Kolmogorov-Smirnov 검정에서 정규 분포를 따랐으며, 방사선학적 및 조직학적 계측치의 통계적 비교는 ANOVA 검정으로 시행하였다 ($p < 0.05$).

8주간의 치유 동안 모든 개체는 특별한 합병증 없이 양호한 치유를 보였다. 방사선학적 및 조직학적 계측 결과, 근심측 치조제 면적에 대한 실험 부위(원심측)의 치조제 면적의 변화량은 대조군과 실험군 간에 통계적으로 유의한 차이가 확인되지 않았으며 (55.41±12.95%, 83.29±24.96%, 73.46±16.59%), 치조제의 면적의 감소가 나타났다. 두 실험군의 결손 부위에 대한 광화 조직 형성은 대조군에 비해 통계적으로 유의미하게 적었다 ($p < 0.05$). 두 실험군 간의 조직학적 및 방사선학적 분석 시 유의미한 차이는 없었다. 술 후 1 주에서, 두 실험군은 대조군과 비교하여 혈관내피세포 성장인자 (Vascular endothelial growth factor, VEGF)와 섬유아세포 성장인자-2 (Fibroblast growth factor-2, FGF-2)의 발현 수준이 증가되었다.

결론적으로 2 벽성으로 파괴된 발치와에서 두 종류의 콜라겐 함유 골이식재의 사용은 제한적인 치조제 보존과 감소된 광화 조직 형성을 보인다.

핵심되는 말: 콜라겐 함유 골이식재, 치조제 보존술, 파괴된 발치와, 발치