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Bio-modification of Infected extraction sockets

Using hyaluronic acid with carrier:

An Experimental Study in Dogs

성견에서 히알루론산과 운반체를 이용한

감염된 발치와의 생활성화

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Abstract

Bio–modification of Infected extraction sockets
Using hyaluronic acid with carrier:
An Experimental Study in Dogs

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Objectives: This experimental study aims to evaluate the efficacy of hyaluronic acid on healing of infected extraction sockets compared with rh BMP–2.

Material and Methods: Both third and fourth mandibular premolars of six beagle dogs were hemisected, and the distal roots were extracted at baseline. Subsequently, combined endodontic–periodontic lesions were induced at the remaining mesial roots. After 4 months, the mesial roots on both sides of the mandible were removed. Four sockets per dog were randomly allocated to 4 groups; Group 1: Control, Group 2: only

Absorbable Collagen Sponge (ACS: carrier), Group 3: 1% Hyaluronic Acid (HA) gel + ACS, and Group 4: rh BMP-2 (recombinant human bone morphogenetic protein-2) + ACS. After three months of healing, the dogs were euthanized for micro-CT, histologic and immunohistochemical analysis. In addition, statistical analysis was performed at 5% significance level using Wilcoxon-Mann-Whitney test.

Results: After the lesion induction period (4 months), communication between the periodontal lesion and endodontic periapical lesion was observed at all remaining mesial roots. Alveolar bone overgrowth was observed in groups 3 and 4, but bone volume density was not significantly different among all groups (P -value >0.05). At the crestal portion, mineralization and osteocalcin expression were higher in groups 3 and 4 than in groups 1 and 2 (P -value <0.05).

Conclusion: Treatment with HA can promote bone formation and improve the wound healing rate comparable to rh BMP-2 in infected extraction sockets.

KEY WORDS

Hyaluronic Acid; Bone Morphogenetic Proteins; Cytokines; Bone regeneration; Tooth Socket; Wound Healing.

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I. INTRODUCTION

The ultimate goal of periodontal treatment is to remove periodontal pathology and restore a healthy periodontium. However, tooth extraction is inevitable in cases of poor prognosis of the tooth or severe bone destruction. Following the tooth extraction, vertical and/or horizontal dimensional alveolar bone change have been reported, as alveolar bone is dependent on tooth presence.¹⁻³

Previous animal studies reporting dimensional change have been performed in fresh extraction sockets.⁴⁻⁸ However, if teeth with a severe inflammatory state are extracted, bone resorption may be more extensive and altered,² and alveolar bone destruction may influence the optimal placement of dental implants.²

Thus, ridge preservation techniques have been performed to prevent alveolar dimensional change.⁹⁻¹² Despite the counteracting properties for alveolar bone resorption, two problems exist when non-absorbable graft particles are used; (i) the biomaterials delays the socket healing process,^{13, 14} (ii) the increasing occupancy of the graft particles might reduce the bone to implant contact (BIC) making an optimal implant stability more difficult to achieve in case the healed site is of lower bone quality.¹⁵ Herein, absorbable cytokines, such as growth factors, may be suggested as an adequate alternative to non-resorbable graft particles to promote healing

dynamics and fill the socket with only pristine bone.

Bone morphogenetic protein-2 (BMP-2) is a representative growth factor having osteoinductive effects and is the only bone graft substitute approved by the United States Food and Drug Administration (FDA). A current systematic review reported that placement of recombinant human bone morphogenetic protein-2 (rh BMP-2) in extraction sockets can reduce the change of alveolar ridge width.¹⁶ However, various clinical side effects of BMP-2 were highlighted by another review article¹⁷; including postoperative inflammation, cervical spine swelling, osteoclast-mediated bone resorption, ectopic bone formation, inappropriate adipogenesis, and tumorigenesis. Due to these adverse side effects, there is a need for a stable growth factor that can be used as a bone graft substitute with an osteoinductive effect similar to BMP-2.

Our previous pilot study showed that application of 1% hyaluronic acid (HA) could improve bone formation and wound healing in infected extraction sockets due to its anti-inflammatory, bacteriostatic, and osteoinductive properties.¹⁸ Bone regeneration was promoted in cases using hydroxyapatite/beta-tricalcium phosphate with HA through alteration of physical and chemical properties of the graft material in an animal study.¹⁹ In addition, other recent animal research has reported that combinations of HA and other graft materials could stimulate osteoinduction.^{20, 21} The objective of this subsequent experiment is to evaluate the efficacy of HA on bone formation in compromised extraction

sockets compared with rh BMP-2.

II. MATERIALS AND METHODS

All research protocols of the current experimental study and our previously published articles^{15, 18} were approved by the Institutional Animal Care and Use Committee of Seoul National University, Korea. Six beagle dogs (weight 10~12kg, age 12~15 months, OrientBio, Seoul, Korea) were used in this research. The sample size was calculated using the data of our previous pilot study.¹⁸ An effect size of 1.8567 was obtained from the mean proportions of mineralized bone in the Control and Test groups (47.80 ± 6.60 and 63.29 ± 9 respectively). A priori power analysis with a significance level of 5% and 80% power indicated that total 6 samples per group was enough in the present study. During this experiment, the dogs were managed in individual metal cages (W900 x D800 x H800 mm) with fixed conditions of relative humidity $50 \pm 10\%$, ambient temperature $23 \pm 2^\circ\text{C}$, light/dark cycle 12/12, and air-condition 12~18 changes/hour. In addition, a standard pellet dog-food diet (HappyRang, Seoulfeed Company, Korea) was fed, and ad libitum access to water was supplied.

This experimental study was performed in accordance with Institute of Laboratory Animal Resources, Seoul National University guidelines. In addition, modified 'Animal research: reporting in vivo experiments (ARRIVE)' guidelines were consulted in the reporting of the present research.²² The outline of the experiment is depicted in Figure 1.

Surgical procedure

Tiletamine/zolazepam(0.1 mg/kg, Zoletil[®], Virbac, Carros, France), xylazine(2.3 mg/kg, Rompun[®], Bayer Korea, Ansan, Korea), and atropine sulfate(0.05 mg/kg, Jeil, Daegu, Korea) were administered to the six beagle dogs intravenously for anesthesia. The protocol to create infected extraction sockets in third and fourth mandibular premolars during the lesion induction period (from baseline to 4 months) was similar to that described in our previous study.¹⁵

In brief, the third and fourth mandibular premolars were hemisected, and distal roots were extracted at both sides. To form a combined endodontic–periodontal lesion at the remaining mesial roots, collagen sponge and black silk soaked with *Porphyromonas gingivalis* (ATCC 33277, 1 x 10⁹/mL) were applied to the periphery of the roots to produce the periodontal lesion, and pathogenic bacteria was injected through the canal of the roots to create endodontic pathology after pulp extirpation at baseline (Fig. 2A, 2B). After 4 months (lesion inducing period), both lesions were observed by periapical radiographic images in all beagle dogs, and inflammatory state symptoms of redness, swelling, and calculi deposition were observed (Fig. 2C, 2D). After the extraction of the mesial roots of third and fourth premolars, the infected extraction sockets were randomly allocated to four groups: Group 1 (Control): no treatment, Group 2 (Carrier): Absorbable collagen sponge (Teruplug[®], Olympus Terumo Biomaterials Corporation, Tokyo, Japan), Group 3: 1% Hyaluronic acid gel (Healon, Pharmacia &

Upjohn, Sweden) + Carrier (collagen sponge), and Group 4: rh BMP-2 (O-BMP[®], Osstem Implant Co., Busan, Korea) + Carrier (collagen sponge) (Fig. 2E, 2F). Periapical radiographic images at the third and fourth mandibular premolars were taken at baseline and 4 months using digital X-ray device (Kodak RVG 6100 Digital X-ray Sensor, Eastman Kodak, Rochester, NY, USA) before removal of the mesial roots. Radiographic analysis was performed by a radiographic analysis system (PiView STARTM 5.0, Informer Technologies, Seoul, Korea). After 3 months (socket healing period) from forming infected sockets, the six beagle dogs were euthanized, and mandibles were separated and sawed for micro-computed tomography and histologic and immunohistochemical examination. (For more detailed explanation about surgical procedures and post-surgery protocol, see our previous studies^{15, 18})

Micro-CT analysis

After euthanizing the dogs, blocks of each premolar site were prepared and sliced. The blocks were scanned on a 0.5 mm aluminum filter at 13.3 μm resolution, 60 kV, and 167 μA on a three-dimensional micro focus computed tomography instrument (micro-CT Sky-Scan 1172[™] Skyscan, Kontich, Belgium), and acquired 2-dimensional (2-D) images were saved as BMP files (2240 x 2240 pixels). Two sagittal images captured 3mm mesial and distal from the extraction socket septum were selected by image analysis software (DataViewer 1.5.2.4 64-bit version, Bruker microCT, Skyscan, Kontich, Belgium). The two images were superimposed,

and net areas were measured on a lattice composed of 100 pixels^{9, 23} (Fig. 3). In addition, the 2-D BMP images were reconstructed to 3-dimensional (3-D) images using the CT analysis software program (CT Analyzer, Skyscan, Kontich, Belgium), and bone volume density (BV/TV, BV: Bone Volume of interest, TV: Total Volume of the volume of interest) in the extraction sockets was measured by the software.

Histologic analysis

The separated blocks including surrounding soft tissue were dissected out with a blade and a low-speed bur. The block samples were decalcified in 10% EDTA solution (Sigma-Aldrich, St. Louis, MO, USA) for 30 days after micro focus computed tomography and were routinely embedded in paraffin solution. The paraffin specimens were sectioned bucco-lingually with a tissue sliding microtome (HM315, Microme, Walldorf, Germany) to obtain 5 µm thick sections. Then, a section randomly selected per every five sections was stained with hematoxylin and eosin. The coronal parts of the extraction sockets in all stained specimens were mainly observed (Fig. 4).

Immunohistochemical analysis

Sectioned samples that were randomly selected were hydrated in water-based descending concentrations of alcohol following deparaffinization in xylene and were reacted with 1 X target retrieval solution (Dako Corp,

Carpinteria, CA, USA) for 1 hour at 65°C to expose antigenic sites. Then, the samples were rinsed with 1 X phosphate buffered saline (PBS, pH 7.4) at room temperature and analyzed using immunohistochemistry after pre-treatment with 1% peroxide-methanol solution for 30 minutes to quench the activity of endogenous peroxidase.

First, the samples were incubated with diluted 1 X PBS including 0.3% Triton X-100, 5% normal horse serum, and 1% bovine serum albumin (BSA, Gibco Invitrogen, Carlsbad, CA, USA) for 1 hour at 37°C to prevent non-specific immune reaction to antigen. The specimens were reacted with diluted solution including primary antibody (rabbit anti-bone morphogenetic protein 2 (1:50), BMP-2, MBS2002350, MyBioSource, MBS2002350, San Diego, CA, USA) and anti-osteocalcin (1:50, OCN, ab13418, Abcam, ab13418, Cambridge, MA, USA) for 20 hours at 4°C and rinsed with 1 X PBS. The sections were then incubated with secondary antibodies (biotinylated anti-mouse IgG (Elite kit, Vector Laboratories, Burlingame, CA, USA) and biotinylated anti-rabbit IgG (1:200, Vector Laboratories, Burlingame, CA, USA)) for 1 hour at 37°C and reacted with mixed liquid (1:200, ABC complex, Vector Laboratories, Burlingame, CA, USA) at the same conditions as used for the secondary antibody reaction. After three rinses with 0.1M PBS for 10 minutes, antibody complexes were visualized by 3',3'-diaminobenzidine-tetrahydrochloride (DAB, Sigma-Aldrich, Oakville, ON, Canada) for 1 to 3 minutes. The tissue sections were observed by light microscope (DMR, Leica, Nussloch, Germany) after dehydrating and cleaning. The area of interest (AOI; 2000

x 1000 μm fixed rectangular form) at the coronal part of the extraction socket was established by histological image analysis software (CaseViewer 2.0 RTM 3DHISTECH Ltd. Budapest, HUNGARY) in all specimens under the same conditions (original magnification x 100) and immuno-positive cells for OCN and BMP-2 in the AOI were counted by two examiners (JJ Kim and Ben Amara H). The values for the number of OCN cells were recorded as the means \pm standard deviation and the inter-examiner reliability of duplicate measurements was evaluated by the Pearson's correlation test (0.969).

Statistical analysis

The 2-D values (Net Area(%)), 3-D values (BV/TV (%)), and OCN cells are presented in Table as mean \pm standard deviation and were analyzed statistically using SPSS version 22 software (IBM Software, Armonk, NY, USA). A non-parametric statistical method was used after rejecting the normality assumption in consideration of the total number of samples. The Wilcoxon-Mann-Whitney test was performed to compare the means of the groups. The significance level was set at 5%. (Table 1)

III. RESULTS

The 24 remaining mesial roots of third and fourth mandibular premolars in six beagle dogs exhibited typical inflammatory signs (gingival swelling, redness, poor oral hygiene, and deep pockets) after 4 months of lesion induction from baseline (Fig. 2C, 2D). In addition, periodontal pathology and endodontic lesions were observed in all mesial roots at 4 months on periapical radiographic images. The 24 infected extraction sockets healed uneventfully after 3 months from the time of extraction.

Micro-computed tomography (Micro-CT) and morphometric evaluation

Alveolar bone growth, destruction and bone volume density were evaluated by micro-CT (Table 1). A positive value of Net Area indicated alveolar bone overgrowth while a negative value represented bone destruction. The Net Area values were -10.74 ± 1.78 % for the Control group, -6.55 ± 9.82 % for the Carrier group, 11.73 ± 4.73 % for the HA group, and 15.94 ± 3.12 % for the rh BMP-2 group. These 2-D values showed alveolar bone destruction in groups 1 and 2 and overgrowth in groups 3 and 4 at the crestal portion (Fig. 3). There were no significant differences between groups 1 and 2 or between groups 3 and 4 (Table 1).

The bone volume density (BV/TV) values are 3-D indicators and were 18.00 ± 6.62 % for group 1, 17.89 ± 6.02 % for group 2, 20.06 ± 6.27 % for group 3, and 20.11 ± 6.64 % for group 4. No significant differences among the 4 groups were found (Table 1). The amount of bone regeneration and destruction was affected by the presence of growth factors (HA, rh BMP-2), although the density of regenerated bone was not.

Histologic evaluation

After 3 months of socket healing, the sockets in all 4 groups, i.e., control, absorbable collagen sponge (ACS), hyaluronic acid (HA), and rh BMP-2 sites, were filled with mineralized bone. However, the control groups (Groups 1, 2) and test groups (Groups 3, 4) showed quite remarkable differences with respect to gross morphological characteristics and various components in new bone.

In the control groups (Groups 1, 2), cortication of bone covering the alveolar crest, which is the coronal part of the extraction socket, was not prominent or mature. The line of cortication was not continuous with adjacent cortical bone (Fig. 4A). Most of the mineralized tissue was fine trabeculae, which consisted of primary osteon and lamellar bone with few reversal lines in high-power view. In the periphery of the newly formed bone, the number of osteoblasts was low (Fig. 4B).

In the test groups (Groups 3, 4), the sockets were characterized by the presence of mineralized bone at a higher density than the control groups; newly mineralized cortical bone that was continuous with the old bone was observed at the coronal part of the sockets. The cortical bone line was smoother than that of the control groups. In addition, overgrowth of alveolar bone at the crestal part was observed in the histological section as well as the sagittal image of micro-CT (Fig. 4C). Furthermore, more osteoblasts were seen at the periphery of the newly formed bone in the test groups than control groups (Fig. 4D). Secondary osteons and various reversal lines were also observed in the test groups (Fig. 4E). In addition, osteoclasts were in their lacunae in the sockets of the test groups (Fig. 4F).

Immunohistochemical and morphometric evaluation

The immunohistochemical reactions for OCN and BMP-2 varied in all groups. It was difficult to evaluate and distinguish the cells stained by BMP-2 as the cells lost their original morphology during slide preparation. Therefore, immunohistochemical evaluation was performed only by the reaction for OCN.

The test groups mostly presented stronger expression of OCN than control groups. In the HA group and rh BMP-2 group, lacunae and newly formed bone matrix were intensely stained by OCN. In addition, osteocytes

and osteoblasts lined at the periphery of the bone trabeculae were strongly stained (Fig. 5).

The number of cells immunopositive for OCN was significantly higher in the test groups than control groups. (There were significant differences between groups 1 and 3, 1 and 4, 2 and 3, 2 and 4). The number of OCN cells was 88.67 ± 43.00 in the control group, 83.00 ± 27.56 in the carrier group, 319.00 ± 138.63 in the HA group, and 281.67 ± 125.74 in the rh BMP-2 group. No significant differences were found between groups 1 and 2 or between groups 3 and 4 (Table 1).

IV. DISCUSSION

The experimental model used in the present study (extraction socket model with induced chronic pathology) was presented in our previous studies^{15, 18} and is suggested to be effective to reproduce clinical situations that can retard the bone healing process and induce severe bone destruction after tooth extraction.^{24, 25} Even though our previous research showed an enhanced bone formation by HA application, the extent was not compared with comparable growth factors such as rh BMP-2 and this was also true for other studies evaluating the effects of HA.^{7, 19-21} Therefore, the objective of this study was to evaluate the efficacy of HA and rh BMP-2 in infected extraction sockets. In addition, the evaluation of an adequate carrier was needed because the effect of HA may be hampered due to its hydrophilicity without a carrier, as with rh BMP-2 which showed poor handling properties as a gel type. Absorbable collagen sponge (ACS), which is highly biocompatible and absorbable concurrent with bone formation and is commonly used as a BMP-2 carrier, was adopted as the carrier of HA in this experiment.^{26, 27}

In spite of its cell-adhesive property and proper plasticity, previous studies have reported that ACS alone is unable to compensate for natural alveolar bone resorption due to its limited capacity for space-maintenance.^{26, 27} The micro-CT morphometric data obtained from the Carrier group in this experiment was not significantly different in

comparison to the Control group as the carrier itself could not prevent bone resorption. However, bone overgrowth beyond the entrance of the extraction socket was observed in the Test groups with HA or rh BMP-2 applied to the sockets. An immune-histochemical study about HA in tooth extraction sockets has indicated that numerous collagen fibers and extensive vessel network were observed in the early healing stage, and that the agent could accelerate bone deposition in the sockets.²⁸ In addition, it was reported that HA stimulated the expression of BMP-2 and osteopontin (OPN) to regulate the healing process of tooth sockets.²⁸ These effects may result in bone overgrowth with the socket's healing potential immediately after extraction,^{7, 19, 28, 29} and HA was intensively transferred with ACS than the gel type used in our previous study.¹⁸

A molecular level study reported that HA enhanced the expression of OCN mRNA and stimulated the production of OCN.³⁰ In addition, HA stimulated alkaline phosphatase (ALP) and runt-related transcription factor 2 (RUNX2) expression on day 14, and OCN expression was intensively increased at day 21, suitable for calcium deposition, in another study.³¹ The other recent study indicated that high molecular weight HA promoted mRNA expression of OCN, ALP, and RUNX-2 and enhanced bone formation.³² These reports are consistent with our immune-histochemical data showing high OCN cells in the Test groups.

Herein, the histological analysis demonstrated that the 'cortical cap' at the entrance of the socket – as described by Lindhe et al. in 2014 –

was entirely corticalized in the Test groups, whereas consisting of a thin trabeculae and woven bone in the Control groups.¹⁴ Because healing dynamics proceed from apical and lateral to marginal regions of alveolar sockets,¹⁴ a complete maturation of the cortical cap suggests a faster healing in the sockets from Test groups in comparison to Control groups. Promoting the healing process using growth factors could be called ‘bio-modification’ because this induction by the growth factors is literally changing the environment and healing potential of the compromised extraction socket into a favorable, dynamic state. In addition, the numerous reversal lines and secondary osteons were observed in the Test groups, similarly to our previous study,¹⁸ and indicate that the enhanced bone forming activity arose from tissue modeling and remodeling.

The present study applied HA with ACS carrier in sockets to hasten socket healing, to accelerate bone formation with normal bone volume density (BV/TV) comparable to that with rh BMP-2, and to resolve infection through its anti-inflammatory and bacteriostatic properties.

The limitation of the present study was the single euthanasia time point. The infected extraction sockets formed over 4 months, and all beagles were sacrificed at the same time, after 3 months of socket healing. The production and degradation of proteins on initial socket healing should be observed at various euthanasia time points, and the healing mechanism of infected sockets may be determined through the immunohistochemistry of diverse cytokines involved in bone formation, in addition to OCN. In

addition, the side effects of HA should be tested despite its good biocompatibility as a component of the extracellular matrix, and a study should be performed with healing time longer than 3 months.

V. CONCLUSIONS

These experimental results suggest that the application of HA with ACS in compromised extraction sockets provides enhanced regenerative efficacy for bone healing comparable to rh BMP-2. Furthermore, it may be suggested that effective guided bone regeneration (GBR) using HA can be expected in challenging GBR cases with adverse bone configuration. Further studies are required to provide insights into the mechanism of action of HA. In particular, there is a need for pre-clinical experiments describing the influence of HA on the healing kinetics and inflammation within infected extraction sockets. Ultimately, clinical researches may be conducted to verify the outcomes for GBR procedures in combination with HA.

VI. TABLES AND FIGURES

Table 1. Micro-CT 2- or 3-D values and the number of OCN cells in immunohistochemical staining in all groups (mean \pm standard deviation)

Groups	N	Net Area (%)	BV/TV (%)	OCN cells
1 (<i>Control</i>)	6	-10.74 \pm 1.78 ^a	18.00 \pm 6.62	88.67 \pm 43.00 ^a
2 (<i>ACS</i>)	6	-6.55 \pm 9.82 ^a	17.89 \pm 6.02	83.00 \pm 27.56 ^a
3 (<i>HA+ACS</i>)	6	11.73 \pm 4.73 ^b	20.06 \pm 6.27	319.00 \pm 138.63 ^b
4 (<i>rhBMP-2+ACS</i>)	6	15.94 \pm 3.12 ^b	20.11 \pm 6.64	281.67 \pm 125.74 ^b
P-value		< 0.05	> 0.05	< 0.05

BV: Bone Volume, TV: Total Volume of the volume of interest, BV/TV: bone volume density.

P-value by the nonparametric Kruskal-Wallis test and Wilcoxon-Mann-Whitney test for each group.

*Different letters (a, b) indicate statistical significance under Bonferroni correction.

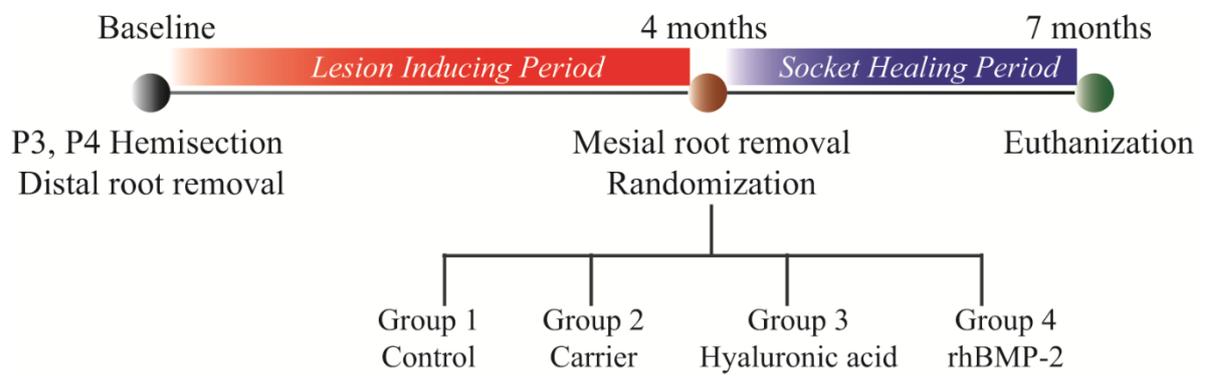


Fig. 1. Outline of the experiment.

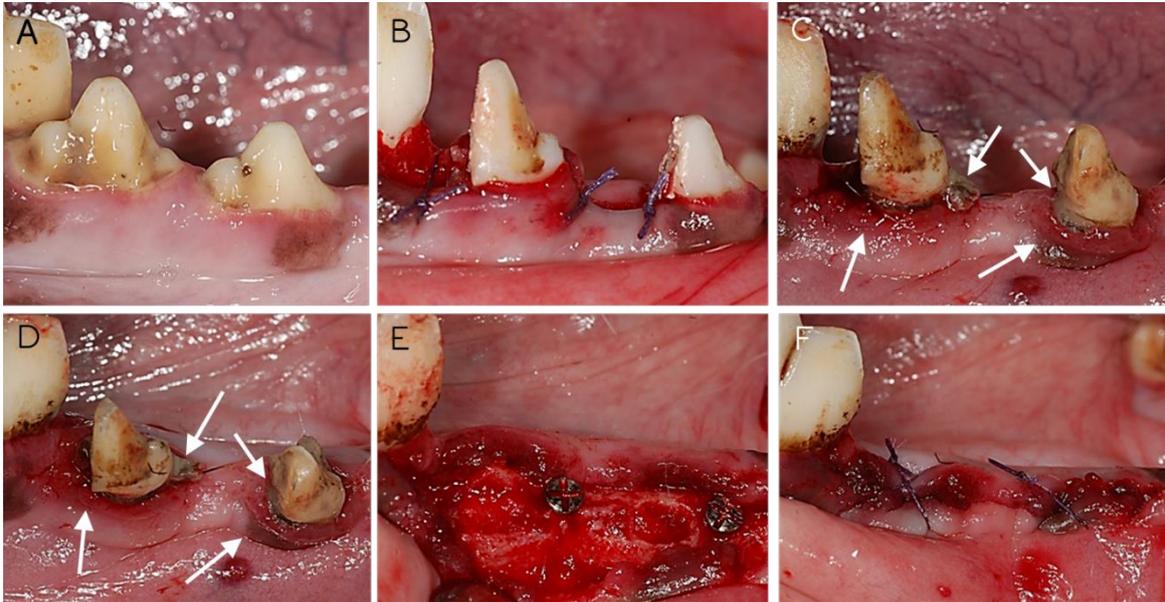


Fig. 2. Clinical photographs representing the entire experimental protocol; (A) initial state before experiment, (B) baseline; induction of a combined endodontic–periodontic lesion, (C, D) 4 months state exhibiting inflammatory signs (gingival swelling, redness, poor oral hygiene, and deep pockets; white arrow) after the lesion inducing period, and (E, F) extraction of the remaining mesial roots and treatment according to group.

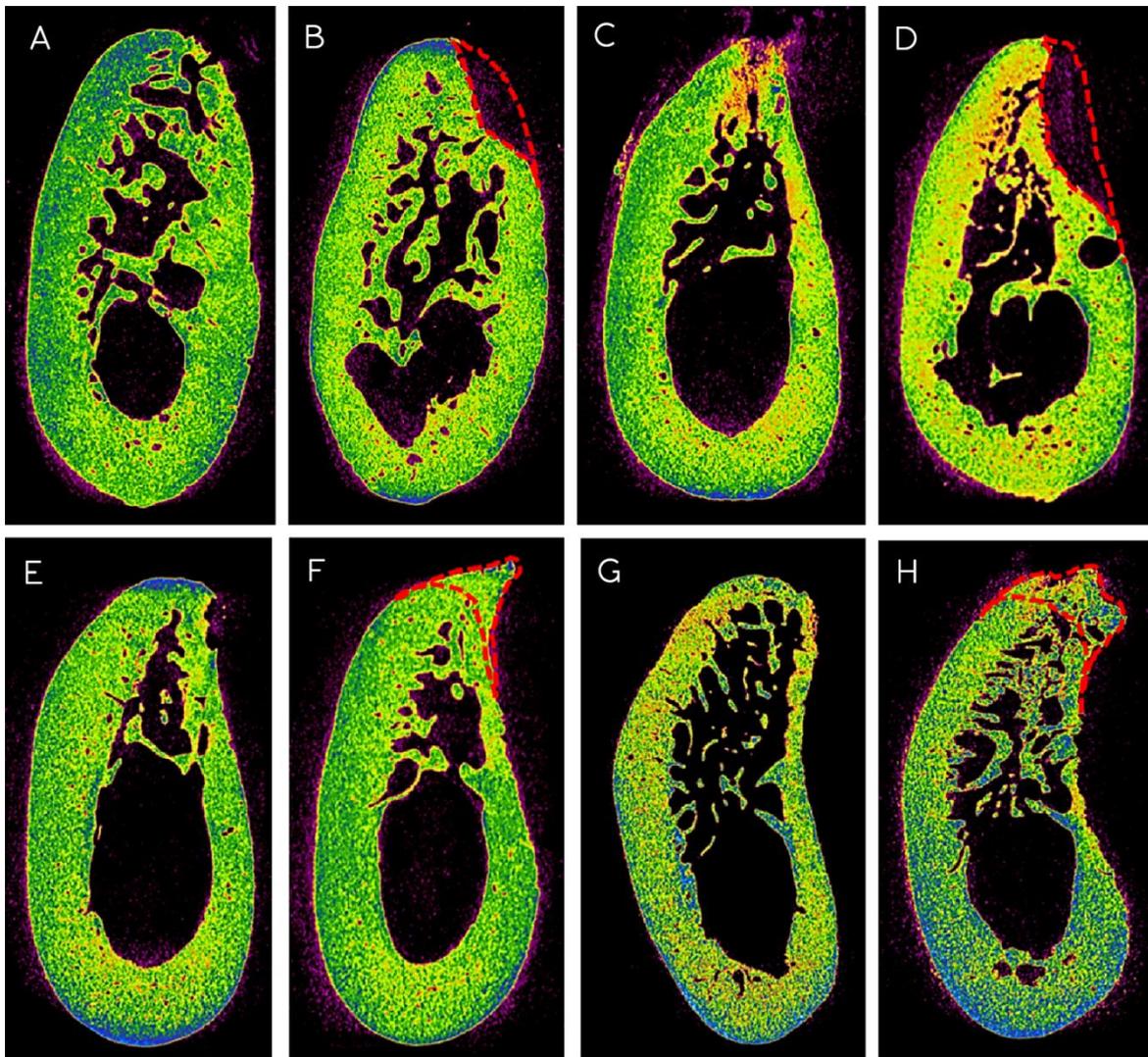


Fig. 3. Representative micro-CT images showing bone growth and destruction in each group; (A, B) Control group/Group 1, (C, D) Carrier group/Group 2, (E, F) HA group/Group 3, and (G, H) rh BMP-2 group/Group 4. The left side image of each pair is the bucco-lingual section on the mesial side 2~3mm from the premolar septum, and the right side image is the section on the distal side 2~3mm from the septum. Each

area enclosed with red dotted line is formed by superimposing the right side image on that of the left and shows the amount of bone destruction (B, D) and growth (F, H).

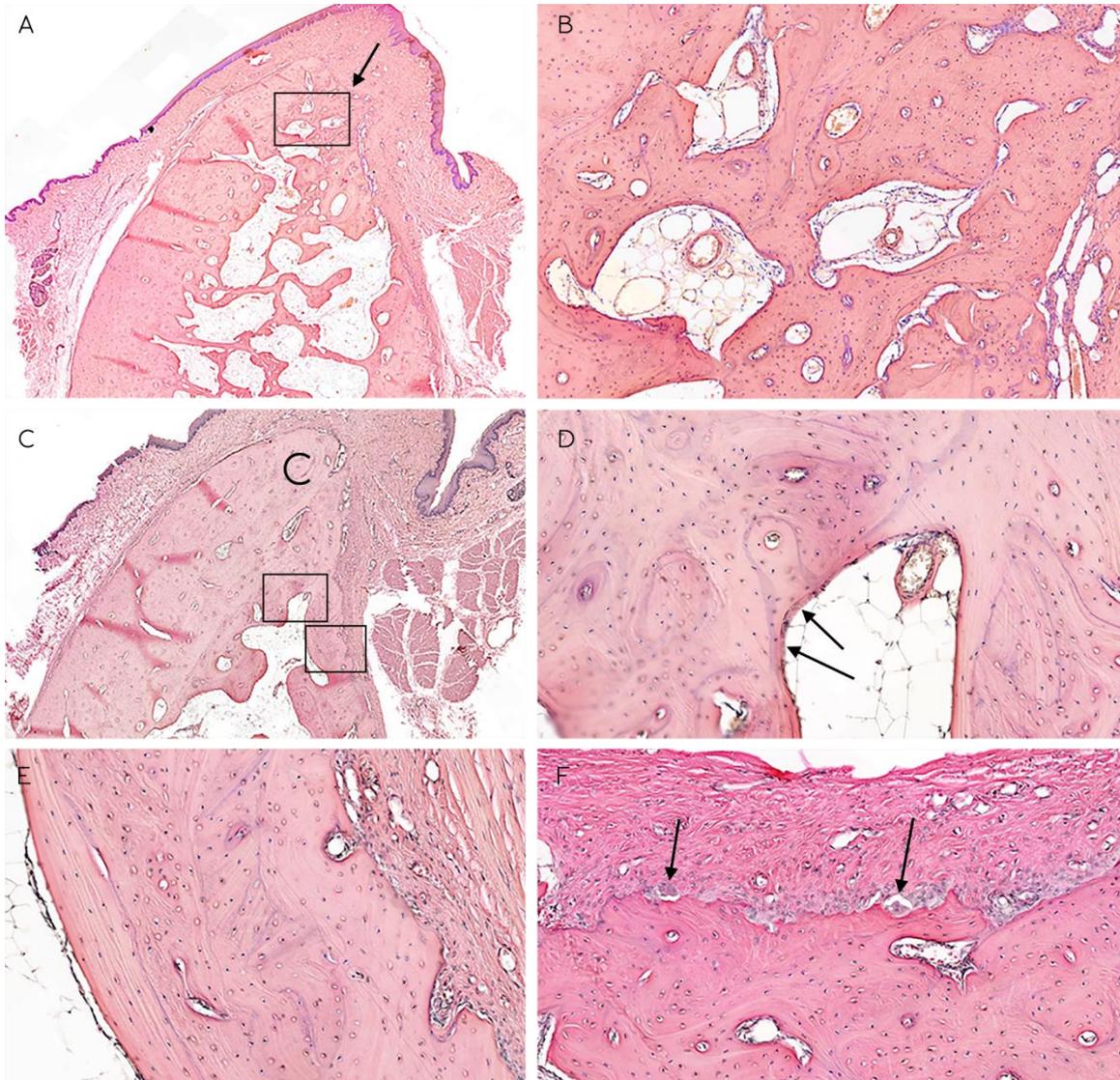


Fig. 4. (A) Overview of the extraction socket of the control group after healing. Note the fine bone trabeculae filling the socket. No cortication is seen on the crest of the alveolar bone (arrow); original magnification x 12.5. (B) Magnification of the rectangle in (A). High power view of the new bone of the control group. Note the fine trabeculae, which consisted

of primary osteon and lamellar bone with few reversal lines in high-power view. Osteoblasts are rarely seen; original magnification x 100. (C) Overview of the extraction socket of the test group (HA group). Note the newly formed cortical bone (C) at the top of the extraction socket, which was continuous with the lingual cortical bone; original magnification x 12.5. (D) Magnification of the upper rectangle in (C). Osteoblasts arranged at the periphery of the new bone (arrows) and secondary osteons; original magnification x 200. (E) Magnification of the lower rectangle in (C). Note the reversal line and secondary osteons; original magnification x 200. (F) Magnification of another HA group. Note the osteoclasts (arrows) at the periphery of the new bone; original magnification x 200.

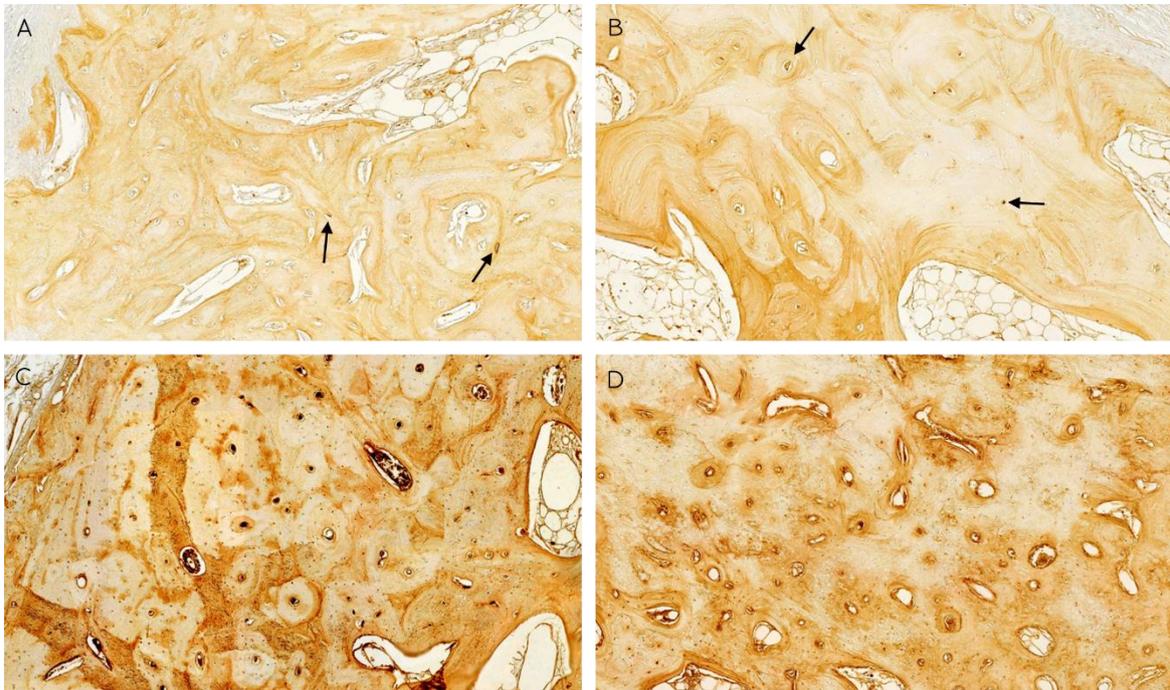


Fig. 5. In the control group (A) and carrier group (B), OCN faintly stained newly formed bone matrix of the coronal part of the extraction socket (black arrow); original magnification x 100. In the HA group (C) and rh BMP-2 group (D), osteocytes, lacunae, and newly formed bone matrix were intensely stained by OCN; original magnification x 100.

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국문 초록

성견에서 히알루론산과 운반체를 이용한 감염된 발치와의 생활성화

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1. 연구 목적

이 연구는 실험 동물 모델에서 감염된 발치와의 치유에 대한 히알루론산의 효과를 rh BMP-2와 비교하여 측정하는 것이 목적이다.

2. 연구 방법

먼저, 6마리 성견들의 양측 3번째, 4번째 하악 소구치에 편측 절단술을 시행하여 원심측을 발치 한다. 그리고, 남아있는 근심측 치근에 근관-치주 복합 병소를 유발시킨다. 4개월의 병소 유발 기간을 가진 후에, 하악 양측 4개의 근심측 치근을 발치하여 감염된 발치와를 만든다. 한 개체당 4개의

발치와를 무작위로 1군은 대조군, 2군은 오직 흡수성 콜라겐 스폰지(운반체)만 적용한 군, 3군은 1% 히알루론산과 흡수성 콜라겐 스폰지 적용한 군 그리고 4군은 rh BMP-2 (recombinant human bone morphogenetic protein-2)와 흡수성 콜라겐 스폰지 적용한 군으로 나누었다. 3개월의 발치와 치유 기간을 가진 뒤에, 6마리의 성견들을 방사선학적, 조직학적, 그리고 면역조직화학적 분석을 위해 희생시켰다. 또한, 윌콕슨-맨-휘트니 검정을 통해 5% 유의수준에서 통계분석을 하였다.

3. 결과

4개월의 병소 유발 기간 후에 남아있는 근심 치관에서 치주병소와 근관병소가 개통된 것을 방사선학적으로 확인하였다. 그룹 3과 4에서 치조골 과성장이 관찰되었고, 골 부피 밀도는 모든 그룹에서 유의미한 차이가 없었다(P -값 >0.05). 또한 치조정 부위에서 골화 정도 및 Osteocalcin 발현 정도가 그룹 1과 2에 비해 그룹 3과 4에서 유의미하게 높은 것이 관찰되었다(P -값 <0.05).

4. 결론

감염된 발치와에 적용된 히알루론산은 rh BMP-2에 상응하는 정도로 골형성 및 창상 치유를 촉진시킬 수 있다.

주요어: 히알루론산, 골형성 단백질, 사이토카인, 골재생, 발치와, 창상치유

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