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Original Article

Acemannan induces rapid early osseous defect healing after apical surgery: A 12-month follow-up of a randomized controlled trial

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Clinical study

Abstract *Background/purpose:* Acemannan is an osteoinductive material. This study's objective was to compare the outcomes of bone defect healing using 3-dimensional images after apical surgery with or without adding acemannan sponges.
Materials and methods: Twenty-two anterior teeth from 9 males and 13 females requiring apical surgery were included in this randomized controlled trial. Post-surgery, the bone defects were randomly divided into three groups: blood clot control, 5-, or 10-mg acemannan sponge groups. CBCT scans were taken immediately (baseline), 3-, 6-, and 12-month post-surgery. Sagittal serial sections (1 mm thick slices parallel to the long axis of the tooth) of the defect image were created. The defect boundary was located and the total bone defect volume (BDV) was calculated from the sum of the volume of the serial defect sections. The bone healing was assessed by the percentage of total bone defect volume reduction (%ΔBDV). The paired t-test and one-way ANOVA were used to analyze the differences within each group and between groups, respectively.
Results: The baseline mean BDV of the control, 5-, and 10-mg acemannan groups were not significantly different ($p > 0.05$). After treatment, the mean BDV for each group was reduced

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in a time-dependent manner. Compared with the control group, the 5- and 10-mg acemannan groups had a significantly greater %ΔBDV (approximately 2- and 1.89-fold) at 3-months post-surgery, respectively ($p < 0.05$). However, at the 6- and 12- month follow-up, the %ΔBDV was not significantly different between the groups.
Conclusion: These data suggest acemannan enhanced early bone healing after apical surgery.

Introduction

Apical surgery is widely used to treat teeth with persistent periapical lesions when endodontic re-treatment is not feasible. After removing the infected periapical tissue and preventing bacterial leakage from the root-canal system, the bone defects undergo self-regenerative healing.[1] However, unfavorable conditions, such as the size or shape of the bony defect and the health status of the patient, can delay the healing process.[2]

It is currently unclear whether adding a regenerative material to the periapical defect after apical surgery enhances bone healing. Some studies reported no difference in bone healing outcomes with or without the use of a regenerative material.[3,4] Several systematic reviews have stated that the addition of regenerative materials did not benefit healing.[5,6] Conversely, other studies have shown that regenerative materials enhance bone healing, indicating that the addition of bone grafts is beneficial to clinical outcomes.[7–10] Because of the disparate methodologies and results in the various studies to date, there is still no consensus as to the benefits of regenerative materials on bone defect healing post-surgery.

The size of the periapical bony defect is an important factor for successful bone healing. Some studies have found a correlation between the bony defect size and the healing duration time, where bigger defects required a longer healing time.[11–14] In contrast, other studies have demonstrated that the periapical defect size may have less influence on healing.[15,16] A less than 5-mm bone defect has a positive prognosis.[11,17] It is currently unresolved whether the use of a regenerative material is advantageous to apical surgery, especially for a larger defect. In addition, apical defect healing is a complicated process that requires both soft tissue (periodontal ligament) and hard tissue (alveolar bone) formation to support the tooth in the jaw. Many researchers are investigating new biomaterials for stimulating both hard and soft periodontal tissues.

Acemannan is a β-(1–4) acetylated polymannose extracted from *Aloe vera* gel. The regenerative effect of acemannan in hard tissue and soft tissue has been intensively studied.[18–23] No fibrous capsule or ankylosis was reported when implanting acemannan sponges in calvaria defects, tooth extraction sockets, pulp capping, and class II furcation defects. Therefore, acemannan is a potential biomolecule for periapical defect healing that requires complex periodontal ligament and alveolar bone regeneration in the periodontium.[23]

Cone beam computed tomography (CBCT) provides 3-dimensional (3D) images of the tooth, soft tissue, and bone that are more accurate than 2-dimensional data obtained from periapical radiographs. Without the superimposed and distorted image often encountered in a 2D image, and a lower radiation dose compared with a medical CT, CBCT has been recommended for Oral and Maxillofacial surgery uses.[24,25] In addition, the CBCT data can be segmented into 3D serial section (coronal, sagittal, and axial planes) that are useful tools for anatomical structure location, diagnosis, treatment planning, follow up, and outcome evaluation. The reliable bone volume measurement via CBCT data assists operators in comparing the results of potential biomaterials on osseous defect healing.[26]

Despite its evaluation for other dental uses, the effect of acemannan on periapical defect healing has not been investigated. Thus, the aim of this study was to evaluate the influence of an acemannan-composed sponge (acemannan sponge) as a graft material in 7.5–15 mm diameter periapical defect healing after apical surgery over a 12-month follow-up period using cone beam computed tomography (CBCT).

Materials and methods

Clinical study

The present study was a randomized controlled clinical trial. The patients were provided with the study details and signed consent forms prior to enrolling in the study. Informed consent was obtained from all individual participants in the study. The patients had the right to decline enrollment and to leave the study at any time.

Study population

The study protocol was approved by the Ethics Committee for Research of the National Hospital of Odonto-Stomatology (NHOS), Hanoi, Vietnam (No. 01/HDDD-BVRHMTWHN) and registered in the Thai clinical trials registry (TCTR20140703002).

Healthy patients aged 18-45 years-old without systemic diseases, non-smoking, or pregnant were included in this study. The patients had a 7.5–15 mm diameter radiographic periapical lesion in an anterior tooth after unsuccessful root canal re-treatment. The eligibility criteria for apical surgery in this study were single-rooted teeth without radiographically overlapping anatomical

structures, dental pulp or tooth root shape abnormalities (such as a calcified root canal, impassable pulp stone, severe root curvature, or constricted canals), restorable tooth, horizontal fracture in the apical third of a root with pulp necrosis, broken instrument or the presence of irretrievable material in the root canal, large and unresolved periapical lesion after root canal re-treatment, or a post and core restored tooth.[27]

Using the results from earlier studies[19,20] and with a type I error of 5% and a power of 0.80, a sample size of 7 subjects for each group was required to demonstrate significant differences between the groups. The sample size of each group was adjusted to 8 considering the possibility of a 10% patient attrition rate.

Acemannan sponge preparation

Acemannan was extracted from *A. vera* gel as previously described.[18,20] Briefly, *A. vera* pulp gel underwent homogenization, centrifugation, precipitation with alcohol, and lyophilization. The white precipitate was characterized by ^{13}C NMR, ^{1}H NMR, and FT-IR, which confirmed that the precipitate was acemannan.

The isolated acemannan was used to prepare 0.5% and 1% (w/v) acemannan solutions. To generate the acemannan sponges, 0.5% and 1% (w/v) acemannan solutions (1 ml) were frozen at −80 °C for 16 h before being lyophilized for 16 h, generating 5- and 10-mg acemannan sponges, respectively. Thus, the sponges were composed solely of acemannan. The sponges were sterilized using gamma irradiation (Thailand Institute of Nuclear Technology, Bangkok, Thailand). The sponges were kept in a desiccator at room temperature until used.

Surgical procedure

All surgical procedures were performed by the same operator. Before surgery, the patients received general physical and oral examinations. The patient rinsed with 0.12% chlorhexidine solution (Kin Gingival, Livar, Spain) for 1 min prior to local anesthesia (2% lidocaine with epinephrine 1: 80,000; Lignospan Special, Septodont Inc., France). A mucoperiosteal flap was created to access the periapical lesion. If necessary, the labial bone was removed with a slow speed round bur (Dentsply, USA) under copious sterile normal saline irrigation to access the lesion. The infected tissue surrounding the root was atraumatically removed by curettage. The excised tissue samples were stored in 10% formaldehyde solution, and sent to the Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University for histopathological evaluation.

After controlling the bleeding, the infected root was resected 2−3 mm from the apex.[13] A 2−3 mm deep retrograde cavity was created on the cut root face using an ultrasonic tip. The root-end filling material (MTA angelus white, Londrina, Brazil) was inserted into the cavity, and excess material removed. A dental curette was used to completely remove infected tissue. Finally, the defect area was irrigated with sterile normal saline to wash out and clean the area.

The defects were randomly assigned into three groups: (A) 5 mg acemannan sponge, (B) 10 mg acemannan sponge, and (C) blood clot-control group using a random group generation program (Excel 2007, Microsoft, Redmond, WA, USA). The patients randomly selected an opaque envelope that was passed to the operator to reveal the assigned treatment. The flap was then repositioned and sutured with a 4.0 non-absorbable suture (Ethicon Inc., Somerville, NJ, USA) in a simple interrupted pattern. A baseline CBCT radiograph was immediately taken.

Each patient was instructed to rinse with 0.12% chlorhexidine solution (10 ml for 1 min. BID for 5 days) and take ibuprofen 400 mg, BID as need for 3 days. The patients were called to ask about any post-surgery complications at day-1 and -3. The patients received appointments for suture removal and clinical evaluation at day-7 post-surgery. The patients were recalled at 3, 6, and 12 months to evaluate the bone healing using CBCT.

CBCT measurement and evaluation

CBCT images were obtained at immediate (baseline), 3-, 6-, and 12-month post-operatively using Planmeca ProMax (Planmeca, Helsinki, Finland) using the following parameters: 96 kV, 5.6 mA, and 12.08 s. The DICOM data were captured and analyzed using Osirix® software (DICOM Osirix imaging software, Pixmeo, Geneva, Switzerland). To standardize the measurements, the long axis of the tooth was set in the labial−lingual plane, and then sagittal serial sections of the defect image with 1 mm thick slices parallel to the long axis of the tooth were created. The total bone defect volume (BDV) was obtained using the following equation:

$BDV_t = V_1 + V_2 + \ldots + V_n\ mm^3 = [(\text{Area of slice}_1 \times 1\text{ mm thick slice}) + (\text{Area of slice}_2 \times 1\text{ mm thick slice}) + \ldots + (\text{Area}_n \times 1\text{ mm thick slice})]$

BDV_t: The total bone defect volume at the designated time (t = immediate, 3-, 6-, and 12-month post-operation) when

V_1: bone defect volume of the first medial section.
V_2: bone defect volume of the second medial section.
V_n: bone defect volume of the last lateral section.

The percentage of total bone defect volume reduction (%ΔBDV) at each evaluation period was[20]

$$\%\Delta BDV_t = [(BDV_{intermediate} - BDV_t)/BDV_{intermediate}] \times 100$$

When t = 3-, 6-, and 12-month post-operation.

Triplicate measurements were performed by blinded oral radiology and endodontic specialists. The intra-rater and inter-rater reliabilities were determined. The radiographs were re-measured by the same evaluators two weeks after the prior examination. The intra-rater and inter-rater reliabilities were 0.91 and 0.894, respectively.

Statistical analysis

The SPSS program version 18.0 (Chicago, IL, USA) was used for statistical analysis. Descriptive analysis was

performed. Comparison of the total bone defect volume and the percentages of total bone defect volume reduction between and within each group were analyzed using One-way ANOVA and Student's paired t-test, respectively. A p-value <0.05 was considered statistically significant.

Results

Twenty-four patients were included in this study (14 females and 10 males). The patients' mean age was 29.8 ± 10.3 years old. Two patients (one each from the 5-mg acemannan and the 10-mg acemannan groups) did not attend the 6- and 12-month follow-ups because of moving out of state. Therefore, 22 patients participated in this study (8 in the control, 7 in the 5 mg acemannan, and 7 in the 10 mg acemannan groups). No patients reported any post-surgical complications.

The radiographic data indicated that in each group, continuous defect healing occurred in a time-dependent manner (Figs. 1—3). The osseous healing initiated from the basal parts to the alveolar parts of the defects, and from the periphery to the center. Spongy bone formation was observed 3-months post-surgery. The acemannan groups demonstrated a more rapid increase in bone formation compared with the control group. Moreover, the new bone that formed in the acemannan and the control groups adjacent to the remaining root surface exhibited periodontal ligament formation with no ankylosis.

The data obtained from the immediate post-operative CBCT indicated there was no significant difference in the mean baseline BDV between the control, 5-, and 10-mg acemannan groups ($p > 0.05$). The baseline BDV of the control, 5- and 10-mg acemannan groups were 195.30 ± 49.49, 241.14 ± 49.01, and $254.21 \pm 58.27\ mm^3$, respectively (Fig. 4A).

At all evaluation time points, the 5-mg acemannan group demonstrated the highest %ΔBDV, while the control had the lowest. At 3-month post-surgery, only the 5- and 10-mg acemannan groups presented a significant reduction in mean BDV compared with baseline ($p < 0.05$, Fig. 4A). The $\%\Delta BDV_{t=3month}$ of the control, 5-, and 10-mg acemannan groups were 28.44 ± 6.61, 56.88 ± 4.77, and 53.93 ± 5.71, respectively. The mean $\%\Delta BDV_{t=3month}$ of the 5- and 10-mg acemannan groups was greater than that of the control group by approximately 2- and 1.9-fold, respectively ($p < 0.05$, Fig. 4B).

At 6- and 12-month post-surgery, each group demonstrated a significant reduction in mean BDV and %ΔBDV compared with baseline ($p < 0.05$). However, the $\%\Delta BDV_{t=6month}$ and the $\%\Delta BDV_{t=12month}$ were not significantly different between the groups ($p > 0.05$). The $\%\Delta BDV_{t=6month}$ of the control, 5-, and 10-mg acemannan groups were 71.57 ± 6.9, 83.96 ± 3.74, and 82.09 ± 6.2, respectively. We found that the defect volume continued to decrease in each group at the 12-month follow-up, the $\%\Delta BDV_{t=12month}$ of the control, 5-, and 10-mg acemannan groups were 89.38 ± 3.54, 96.59 ± 1.05, and 95.22 ± 1.48, respectively.

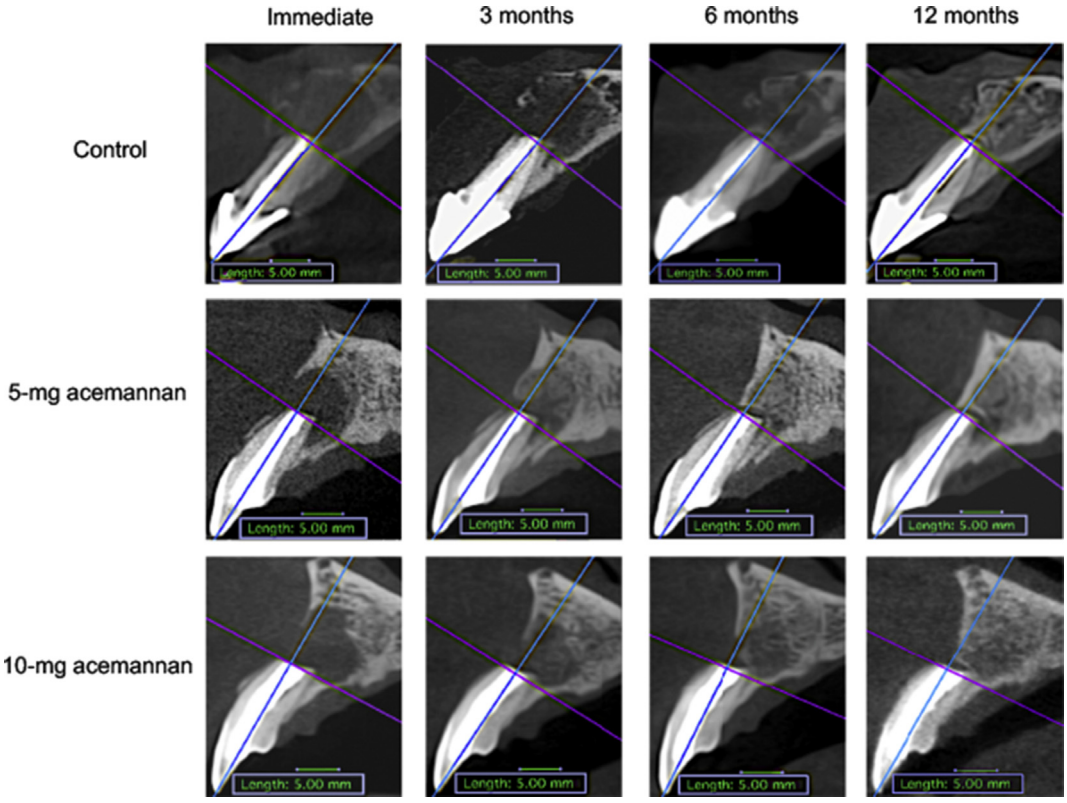

Figure 1 Representative sagittal CBCT images of the control, 5-mg acemannan, and 10-mg acemannan groups at immediate, 3-, 6-, and 12-month post-surgery. Bar = 5 mm.

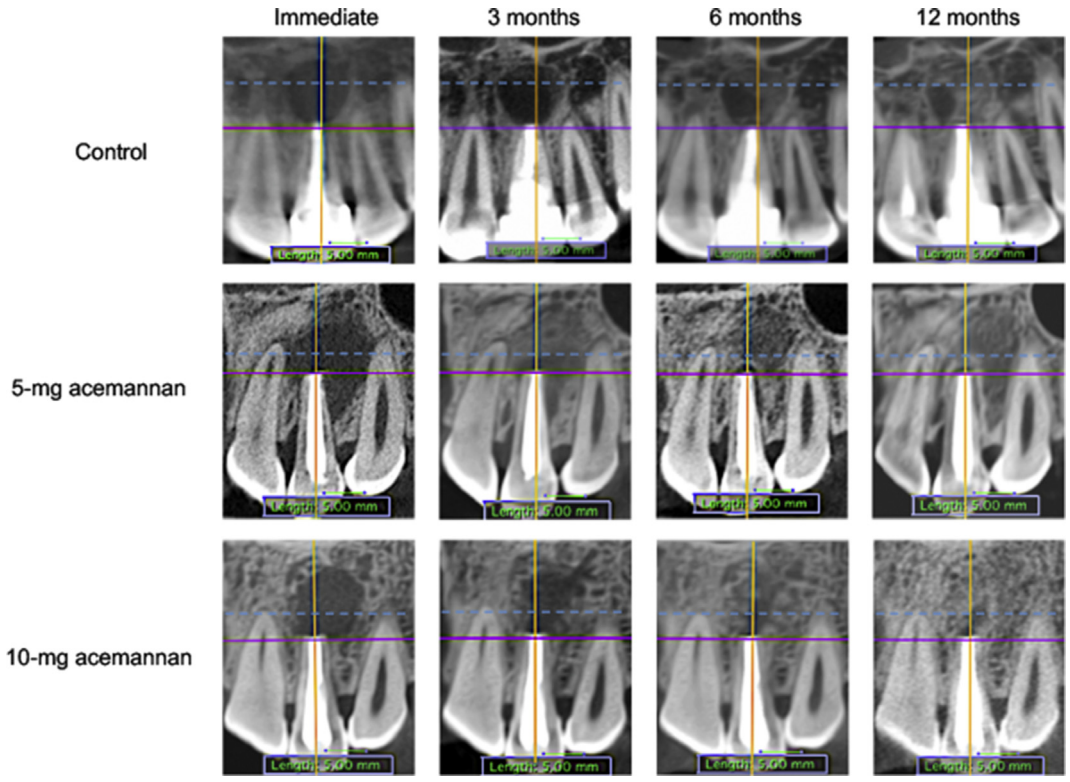

Figure 2 Representative coronal CBCT images of the control, 5-mg acemannan, and 10-mg acemannan groups at immediate, 3-, 6-, and 12-month post-surgery. The dashed line in the coronal view demonstrates the plane of the axial view at the maximum diameter of the defect. Bar = 5 mm.

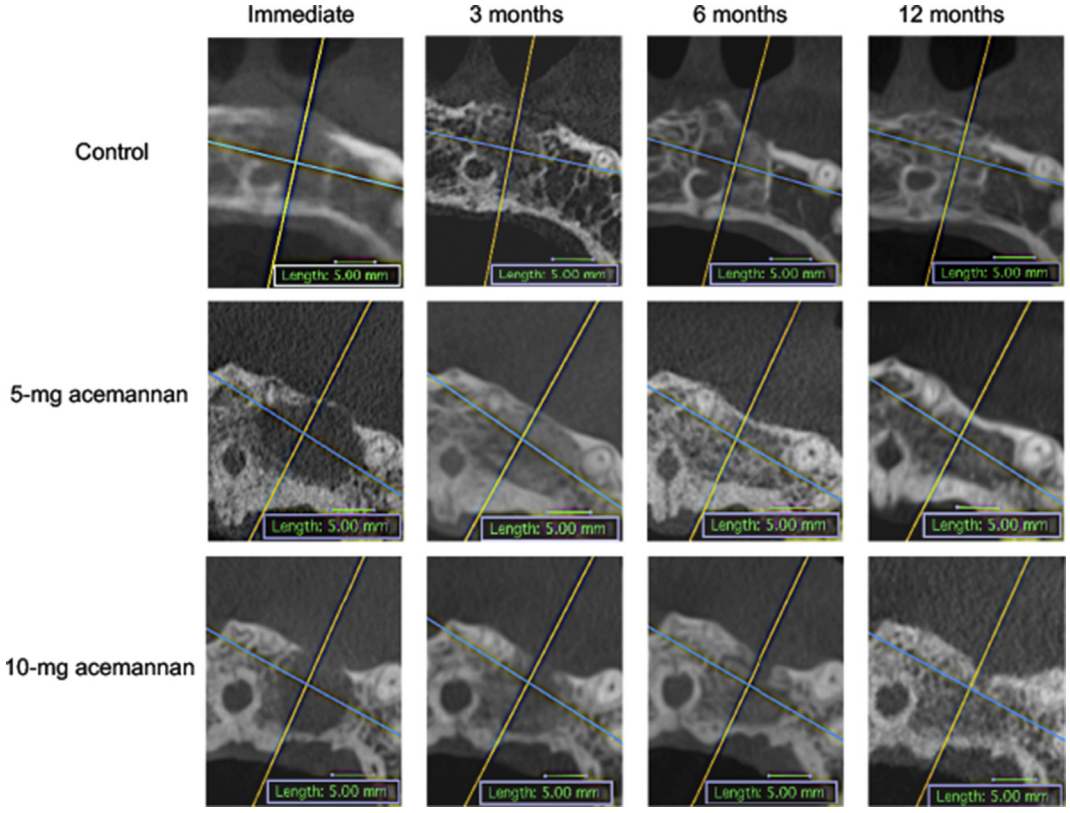

Figure 3 Representative axial CBCT images of the control, 5-mg acemannan, and 10-mg acemannan groups at immediate, 3-, 6-, and 12-month post-surgery. Bar = 5 mm.

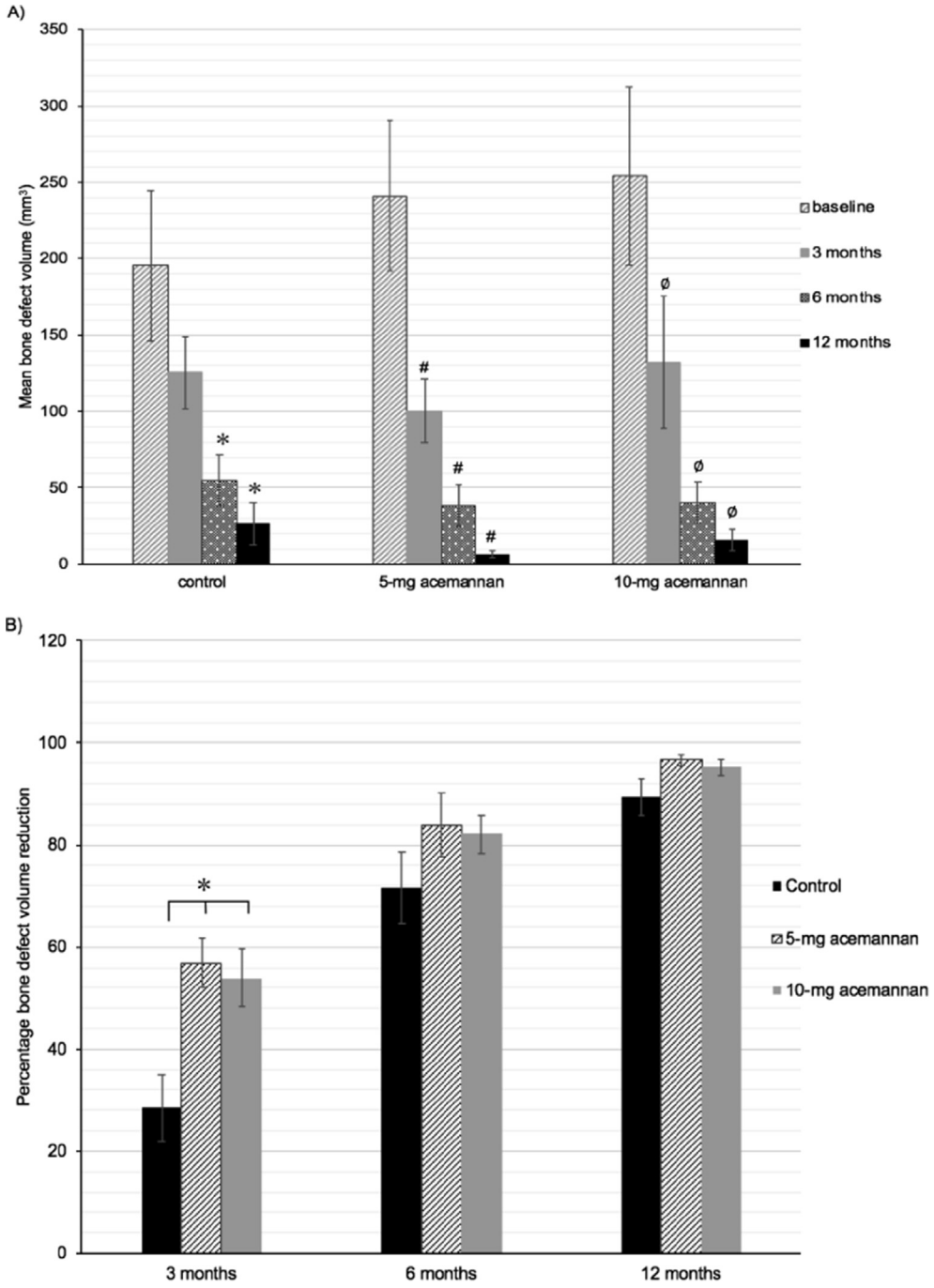

Figure 4 A) The mean ± SE total bone defect volume of the control, 5- and 10-mg acemannan groups at baseline, 3-, 6- and 12-month post-surgery. *, #, and ∅ indicate a significant difference between the mean bone defect volumes at 3-, 6-, and 12-month post-surgery and the baseline, respectively. B) The mean percentage of total bone defect volume reduction (%ΔBDV) of the control, 5-, and 10-mg acemannan groups at 3-, 6-, and 12-month post-surgery. * indicates a significant difference in %ΔBDV in the 5- and 10-mg acemannan groups compared with the control at the evaluation point ($p < 0.05$).

Discussion

This prospective clinical trial used CBCT analysis to investigate the benefit of acemannan sponges on bone repair after apical surgery. The acemannan sponges were biocompatible and demonstrated an early osteoinductive effect in periapical defect healing.

There are several techniques to assess bone healing post-apical surgery, including periapical radiographs, CBCT, and histopathology. The CBCT is considered a clinically effective method for 3D evaluation prior to apical surgery and long-term follow-up.[1,24,25,28] CBCT provides a precise volumetric measurement of the periapical bone defect, which is comparable to that of micro-CT.[26,28] The Osirix

software has been recommended for providing reliable and reproducible volumetric measurements of CBCT data.[24,29–31]

The 3D data indicated that the bone healing was initiated from the wall of the defect, beginning in the basal portion rather than the alveolar portion of the defect, and starting from spongy bone to compact bone formation. The high vascularization at the basal portion of the jaw bone may explain this observation. The concentric growth center of new bone co-localizes with blood vessels in the spongy bone, which is distant from the compact bone margin. In addition, the spongy bone must fill the defect before compact bone formation occurs. Cortical plate regeneration takes a longer time compared with spongy bone formation, and occurs as the last phase of bone healing.[32] Therefore, the cortical plate should be preserved as much as possible during the surgery to reduce healing time, and the patient should avoid placing heavy occlusal loading on the tooth for the first six months post-apical surgery.

Similar to collagen and chitosan, acemannan generates a degradable, 3-dimensional interconnected sponge by preparing it in solution, followed by lyophilization. The degradation time of an acemannan sponge is approximately 2–3 months.[23] Therefore, the blood clot was selected as the control in this pilot study. However, a clinical comparative study between acemannan and cancellous xenografts in treating periapical lesions is ongoing.

In the present study, the use of 5- and 10-mg acemannan sponges was determined by calculating the ratio of the acemannan sponge concentration to the osseous defect volume in our previous study.[20] The data obtained in the present study suggest that both 5- and 10-mg acemannan sponges are effective for bone healing in 7.5–15 mm diameter sized defects. Based on the mean percentage of defect volume reduction and safety, the optimal concentration should be the 5-mg acemannan sponge for this range of defect volumes.

Although the control and the acemannan groups all demonstrated continued healing at each observation time, the acemannan groups had greater healing rates than that of the control group. At the 3-month follow-up, the defect volume reduction in the acemannan groups was more than 50%, while that of the control group was only 28%. Placing acemannan sponges in the extraction socket resulted in increased radiodensity of the tooth socket at 3-months after surgical removal of wisdom teeth.[20] These findings suggest that acemannan accelerates early bone healing.

Although our results indicated that acemannan accelerates bone defect closure, the specific underlying mechanism of how acemannan impacts bone formation has not been identified. Acemannan sponges have an interconnected 3D structure, and remain in the body for several weeks.[23] The sponge absorbs the blood and serum to form a blood clot, becoming a temporary scaffold for cell attachment, growth factor reservoir, and extracellular matrix deposition.[20,23] Acemannan also induces osteoblast progenitor proliferation and differentiation, and mineral deposition.[19] Moreover, upregulation of BMP-2 and -4, VEGF, and bone matrix proteins secretion have been observed.[19,21]

Another advantage of acemannan is its biocompatibility. We did not receive any reports of post-surgical complications in the acemannan sponge groups. No fibrous capsule or chronic inflammatory cells were detected in the tooth sockets, furcation defects, or calvarial defects that received acemannan sponges.[18,20,23] In addition, these other studies showed that acemannan sponges were no longer present in rodent tooth sockets or calvarial defects 1-month post-surgery, and canine furcation defects 2-month post-surgery.

From our data, the 5 mg acemannan group demonstrated a superior %ΔBDV than that of 10 mg acemannan group at all evaluation time points. Due to the limitations of the study, an exact explanation to the superior effect on osseous defect healing could not be stated. One possibility is that 5 mg acemannan is the optimal concentration for 7.5–15 mm diameter periapical lesions. The immunomodulatory and anti-inflammatory effects of acemannan have been reported.[34–36] Optimal immunomodulation accelerates the inflammatory reaction through macrophage activation and the release of tumor necrosis factor, IL-1,-6,-8 and interferon to heal and repair tissue. A higher concentration of acemannan could alter the immunomodulatory activity resulting in decreased healing efficiency compared with the optimal concentration.

Following the quality guidelines for endodontic treatment from the European Society of Endodontology,[27] the outcome of bone defect healing using acemannan sponge was assessed at 12 months post-surgery. A longer observation time of up to 5-year post-surgery would clearly confirm the efficiency and safety of acemannan sponge use.[33] In conclusion, our results suggest that acemannan sponge is an osteoinductive biomaterial that can be safely used in apical surgery to enhance early osseous defect healing.

Declarations of interest

The authors have no conflicts of interest relevant to be this article.

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