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Original Article

Acemannan-induced tooth socket healing: A 12-month randomized controlled trial

Ngoc Bao Vu [a], Vannaporn Chuenchompoonut [b], Pornchai Jansisyanont [c], Polkit Sangvanich [d], Thanh Ha Pham [e], Pasutha Thunyakitpisal [f*]

[a] Dental Biomaterials Science Program, Graduate School, Chulalongkorn University, Bangkok, Thailand
[b] Department of Oral Radiology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[c] Department of Oral Surgery, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[d] Department of Chemistry, Faculty of Sciences, Chulalongkorn University, Bangkok, Thailand
[e] Department of Implantology, Hanoi National Hospital of Odonto-Stomatology, Hanoi, Viet Nam
[f] Research Unit of Herbal Medicine, Biomaterial, and Material for Dental Treatment, Department of Anatomy, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

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KEYWORDS
Acemannan;
Biomaterial;
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Tooth socket healing

Abstract *Background/purpose:* Natural compounds have become alternatives for bone regeneration. Acemannan, the main polysaccharide extracted from *Aloe* vera, has been demonstrated as a promising osteoinductive material *in vitro* and *in vivo*. This clinical study investigated the effect of acemannan on tooth socket healing.
Materials and methods: Thirty-five otherwise healthy patients, 18–25 years old and diagnosed with horizontal or vertical partial impaction of the lower third molars, were enrolled in this randomized controlled trial. After removing the teeth, the sockets randomly received one of the following treatments: spontaneous blood-clotting (control), 20 mg acemannan sponge, or 50 mg acemannan sponge. Cone-beam computed tomography of the mandible was performed immediately (baseline), and at 3-, 6-, and 12-months postoperatively; the data were analyzed using the OsiriX MD program. Bone healing in the socket was determined measuring the socket volume. One-way ANOVA was used to analyze the differences within each group and between groups.
Results: Thirty-five patients with 43 partially impacted lower third molars participated in this study. No patients exhibited alveolar osteitis or secondary infection. Compared with baseline, all groups showed significant reduction in socket volume at all observation time-points ($p < 0.05$). The 50 mg acemannan group had a significantly greater reduction in socket volume compared with the control at all postoperative time-points ($p < 0.05$). The 20 mg group had a significantly greater reduction in socket volume compared with the control at 3-months post-operatively ($p < 0.05$).

* Corresponding author. Research Unit of Herbal Medicine, Biomaterial, and Material for Dental Treatment, Department of Anatomy, Faculty of Dentistry, Chulalongkorn University, Henri-Dunant Rd, Patumwan, Bangkok, 10330, Thailand. Fax:+66 2 218 8870.
E-mail addresses: pthunyak@yahoo.com, Pasutha.T@chula.ac.th (P. Thunyakitpisal).

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Conclusion: We conclude that acemannan increases bone healing at 3-, 6-, and 12-months after removal of partially impacted mandibular third molars.

Introduction

Impacted third molar extraction is a common procedure in oral surgery.[1] After extraction, the remaining alveolar bone at the socket undergoes remarkable resorption, resulting in poor periodontal attachment at the adjacent second molar.[2] To prevent this resorption, bone grafting in the socket is performed to preserve residual alveolar bone and stimulate socket healing. However, the efficacy of bone grafting in the tooth socket after extraction remains unresolved.[3,4]

Bone regeneration consists of 3 overlapping sequencing phases: inflammation, formation, and remodeling.[5] Inflammation is the initial phase in bone healing and regeneration. Injured cells release cytokines to recruit and activate neutrophils and macrophages to the injured tissue.[6] After killing the bacteria and removing foreign bodies and cell debris, macrophages release growth factors for the next formative phase.[7] Osteoprogenitors and osteoblasts undergo proliferation, differentiation, and mineralization. Osteoblasts release growth factors, produce extracellular matrix, and deposit mineral.[5,8]

An autogenous bone graft is regarded as the optimum method for bone regeneration because it promotes osteogenesis, osteoinduction, and osteoconduction. However, autogenous grafts require a donor site and additional surgery, which can result in patient morbidity. Consequently, many studies are being conducted to find alternative biomaterials that promote bone healing without this disadvantage. There has been a growing interest in natural compounds for bone regeneration, because these compounds provide affordable, non-invasive options to a larger patient population.[9] Studies have demonstrated the positive outcomes of plant extracts on bone healing through either of two mechanisms: osteoconductivity, which refers to the support for new bone ingrowth, or osteoinductivity, where a material stimulates cells to differentiate into osteogenic cells.[10]

Aloe vera (*Aloe barbadensis* Miller) is well known for its effect on wound healing. Acemannan, or â-(1,4) acetylated polymannose, is a major polysaccharide extracted from *A. vera* parenchyma.[11–13] Acemannan induces cell proliferation, growth factors and extracelluar matrix synthesis, and mineral deposition in bone marrow stromal cells, periodontal ligament cells, and dental pulp.[14–16] Acemannan-containing sponges demonstrated osteoinductive activity in a rodent tooth socket model, and acemannan accelerated the formation of new alveolar bone, cementum, and periodontal ligament in canine class II furcation defects.[15] Clinically, acemannan enhanced the healing of aphthous ulcers and reduced the incidence of alveolar osteitis.[17,18] Jansisyanont et al. reported an increase in tooth socket radiodensity in acemannan-treated patients at 3-months postoperatively; however, that study did not confirm the long-term osteoinductive effect of acemannan. Moreover, their findings were based on two-dimensional, rather than three-dimensional (3D), radiographs.[19]

In this study, we hypothesized that acemannan enhances socket healing after mandibular third molar extraction at three, six, and twelve months postoperatively. Cone beam computed tomography (CBCT) was used for 3D evaluation at each time point.

Materials and methods

Acemannan extraction, characterization, and sponge preparation

A. vera was provided by a local herbal supplier in Bangkok, Thailand. After verifying its identity, a specimen was deposited in the Museum of Natural Medicines, Faculty of Pharmaceutical Sciences, Chulalongkorn University (Bangkok, Thailand). Acemannan was extracted and characterized as described previously.[20] Briefly, *A. vera* gel was homogenized, centrifuged, and precipitated in cold ethanol. The precipitates were collected and characterized using ^{13}C NMR and ^{1}H NMR spectroscopy, the results of which were comparable with those of previous studies, confirming isolation of the polysaccharide acemannan.[21]

Acemannan (20 or 50 mg) was dissolved in one ml sterile distilled water and lyophilized for 24 h to generate 20 or 50 mg acemannan sponges. These sponges were then sterilized by gamma irradiation (Thailand Institute of Nuclear Technology, Bangkok, Thailand) and kept under dark and dry conditions at room temperature until use. Godoy et al., revealed that radiation-sterilized acemannan sponges retain their characteristics and bioactivity.[22]

Study population and inclusion criteria and exclusion criteria

This randomized controlled clinical trial was approved by the Ethics Committee for Research, National Hospital of Stomatology, Hanoi, Vietnam (clinical trial no. 339/QD-BVRHMTW) and registered in the Thai clinical trials registry (TCTR20140701001). Non-smoking, non-pregnant, and systemically healthy patients, 18—25 years of age, were referred to the Department of Maxillofacial Surgery for surgical removal of lower impacted third molars with a mesioangular or vertical impaction (based on Winter's classification), position A or B, and class I or II (based on the Pell and Gregory classification).[23,24] Patients were excluded if they were planning to become pregnant within the next twelve months,

taking any medications that may affect socket healing, or were unable to participate for the entire duration of the study. No periodontal disease, abscess, or periapical pathology was detected in the extracted teeth. All patients provided informed consent and agreement prior to participation in the study. G* Power 3.1.9.2 for Windows 10 was used to determine the necessary sample size at 80% power. Assuming a dropout rate of 20% during the study, 42 subjects were required to demonstrate significant differences among the three groups.[25] Fig. 1 summarizes the flow of our study.

Surgical procedure and masking

All surgical procedures were performed by the same surgeon. Before extraction, patients underwent general physical and clinical examinations. Briefly, each patient was given a 0.12% chlorhexidine mouth rinse (Peridex; 3 M ESPE, St. Paul, MN, USA) for 1 min, followed by local anesthesia (inferior alveolar nerve block and local infiltration) consisting of 2% lidocaine hydrochloride with adrenaline (1: 100,000; Septodont, Lancaster, PA, USA). A mucoperiosteal flap was created from the mesial papilla of the adjacent second molar to the anterior border of the mandibular ramus. To expose the impacted tooth, minimal covering bone was removed using a round bur placed on a low-speed straight handpiece under copious saline irrigation. If necessary, the tooth was sectioned and extracted atraumatically. After tooth removal, the socket was debrided and irrigated with sterile normal saline.[26]

The subjects were randomly assigned to 1 of 3 groups: spontaneous blood clotting (control), 20 mg acemannan sponge, and 50 mg acemannan sponge groups. The numbers for randomization were generated for each subject using random allocation software (developed by M. Saghaei, MD., Department of Anesthesia, Isfahan University of Medical Sciences, Isfahan, Iran). Each patient was instructed to show the number to the surgeon during the irrigation step. Acemannan sponges were applied to the sockets in the test groups; the sockets were allowed to heal spontaneously in the control group. The flaps were repositioned and sutured with a 4.0 nonabsorbable suture (Ethicon Inc., Somerville, NJ, USA) in a simple interrupted pattern. The patients were instructed to bite a piece of gauze for at least 30 min. Baseline CBCT scans were taken immediately (Rayscan Symphony; Ray Company, Gyeonggi-do, South Korea).

Patients were prescribed 0.12% chlorhexidine solution (rinse with 10 ml for 1 min, twice per day for 7 d) and ibuprofen 400 mg (twice per day for 3 d).[27] The patients were contacted by telephone to assess any postoperative complications on days 1 and 3. One week postoperatively, the patients returned for a clinical examination and suture removal. To assess the rate of bone healing, the patients returned to the clinic at 3-, 6-, and 12-months postoperatively.

CBCT and data analysis

Each participant underwent CBCT at baseline and 3-, 6-, and 12-months postoperatively using standard parameters

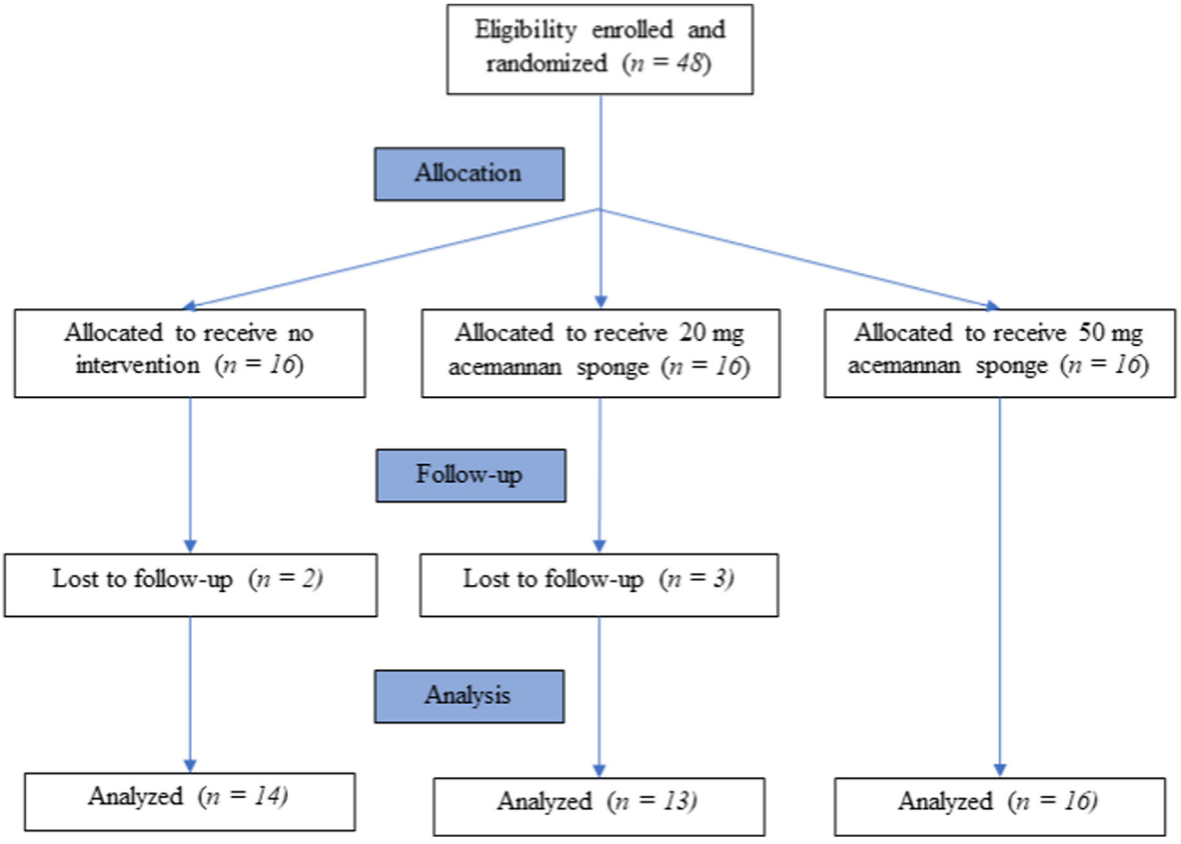

Figure 1 Study flowchart.

(90 kV, 10 mA, and 19.5 s of exposure). The CBCT data were masked by a third party before evaluation and were analyzed by two trained investigators (one maxillofacial surgeon and one oral radiologist) blinded to the treatment.

The OsiriX MD program (Pixmeo, Bernex, Switzerland) was used for data measurement and analysis.[28] To optimize visualization, images were magnified 5x to delineate the bone structure at high resolution. Bone formation in the socket was determined by measuring the socket volume. To standardize this measurement, the sagittal plane parallel to the long axis of the adjacent second molar and anterior border of the ramus was defined. The socket height was then determined from the distal cementoenamel junction of the adjacent second molar to the lowest socket apex, to locate the boundary of the examined area. Subsequently, the investigated area was segmented axially into slices 0.5 mm thick (Fig. 2A). The outline of the socket was selected as the region of interest (ROI) in each slice. The surface area and volume of each slice were measured (Fig. 2B). The sum of all slice volumes was recorded as the total socket volume. The socket volume was calculated using the following formula:

$$V = \{ROI^1 \times T\} + \{ROI^2 \times T\} + ... + \{ROI^n \times T\}$$

V = total volume of the socket (mm^3)

ROI^n = region of interest (mm^2) of slice number n

T = thickness of each slice (0.5 mm).[29]

The percentage change in socket volume (%ΔVt) from baseline to t months was calculated using the following formula:

$$\%\Delta Vt = [(V_0 - Vt) \times 100] \; / \; V_0$$

t = 3, 6, or 12

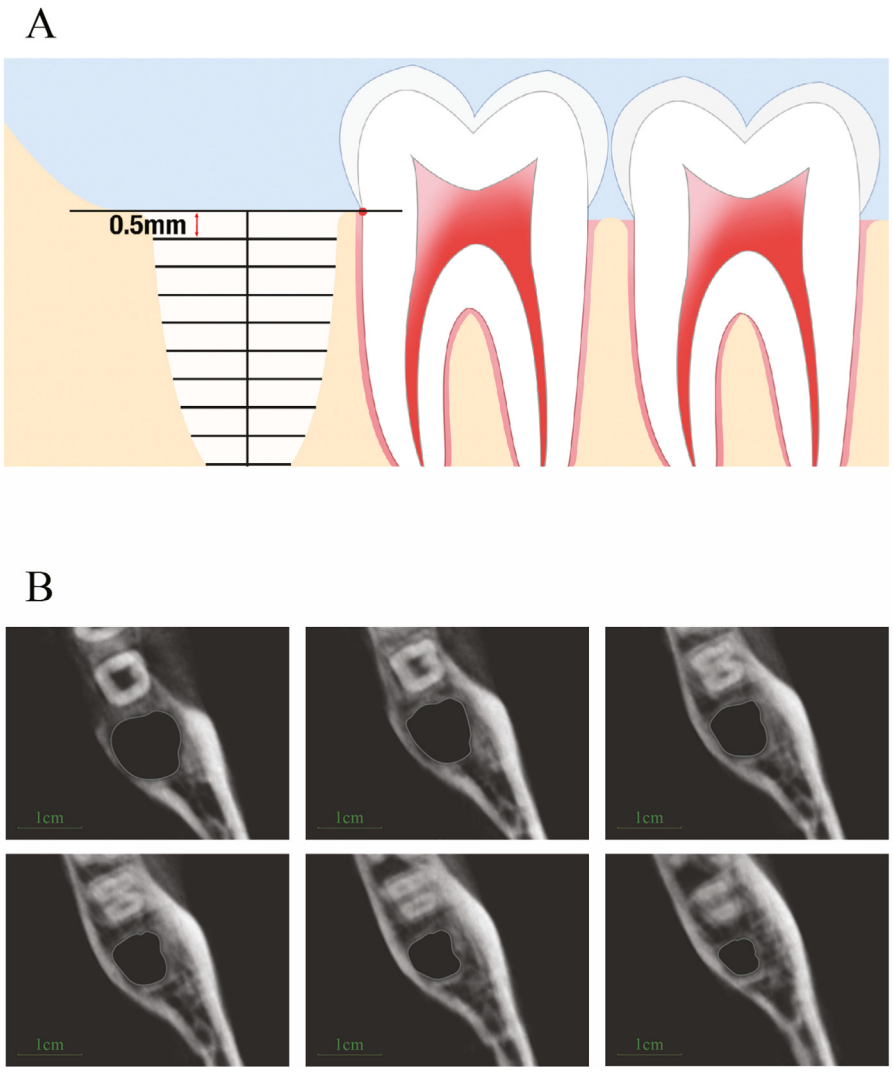

Figure 2 Illustration and radiographic image (5x magnification) of the tooth socket. (A) Socket height was measured from the distal cemento-enamel junction of the adjacent molar to the socket apex of the impacted tooth to establish the examination area. (B) Representative CBCT axial images of the socket. The region of interest (grey line) is the outline of the tooth socket.

V_0 = socket volume immediately after surgery (baseline)

V_t = socket volume at t months after surgery

The ROI of each slice was recorded as the mean of six repeated measurements. The data were re-examined by the same investigator two weeks after the final evaluation.

Data analysis

Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including the means, standard deviations, and standard errors, were determined. Comparisons of the mean socket volume within each group and the mean change in socket volume between groups were analyzed by one-way analysis of variance with Dunnett's post hoc test. Differences with $p < 0.05$ were considered statistically significant.

Results

Thirty-nine patients (22.6 ± 1.9 years old; 26 females and 13 male) with 48 lower third molars (nine bilateral and 30 unilateral) participated in this study. The number of

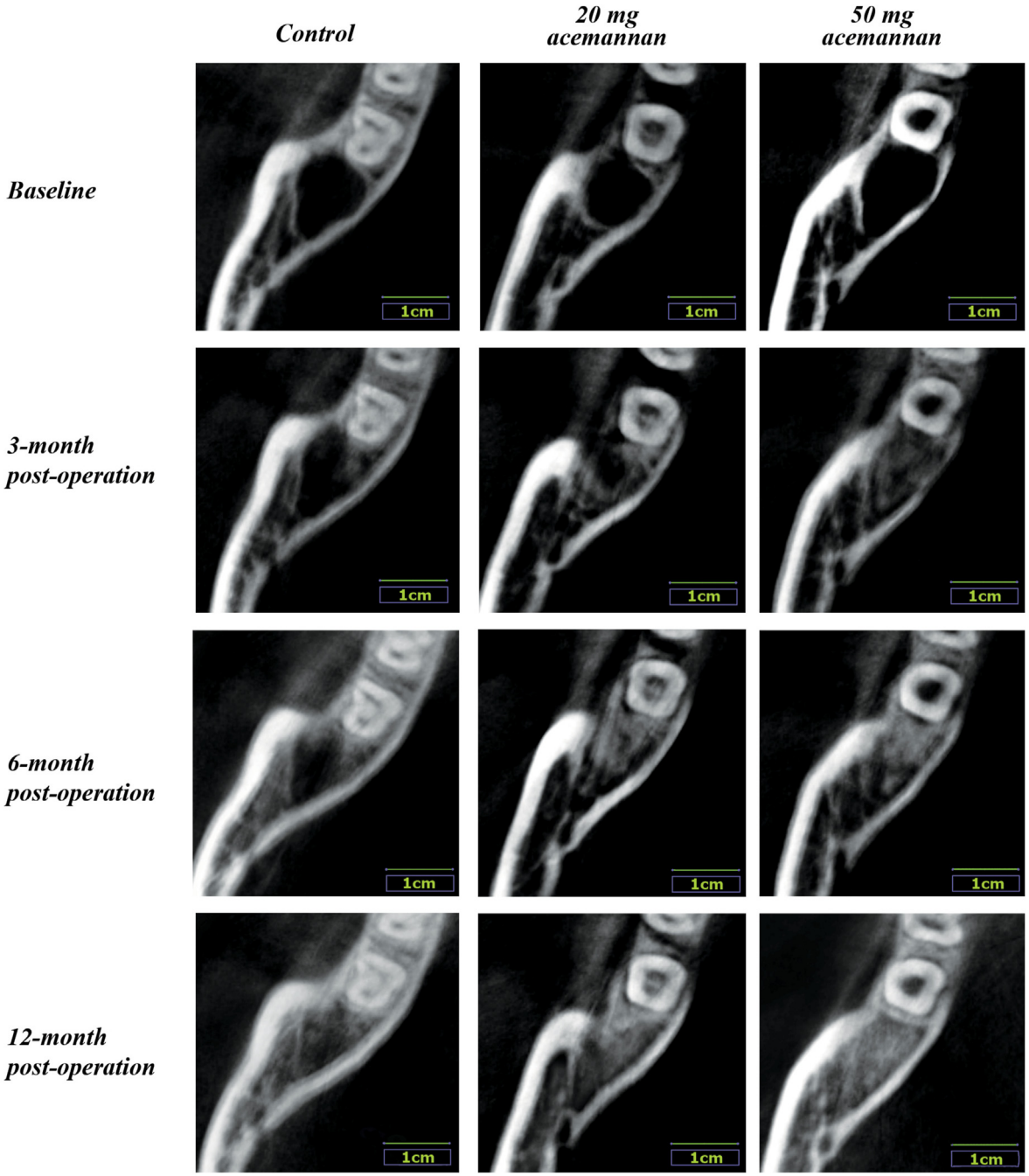

Figure 3 Representative radiographic images of the tooth socket: axial views of the control, 20 mg acemannan, and 50 mg acemannan groups at baseline and three, six, and twelve months postoperatively. Images were generated using the OsiriX MD Program.

mesioangular and vertical impaction were 30 and 18, respectively, which were divided equally into each group (10 mesioangular and six vertical impactions per each group). No postoperative complications, such as dry socket or secondary infection occurred. Pain and swelling at the extraction site were only reported during the first three days postoperatively. Five teeth (2 in the control group and 3 in the 20 mg acemannan group) were lost to follow-up because they moved out of the city. Thus, the final numbers of subjects in the control, 20 mg acemannan, and 50 mg acemannan groups were 14, 13, and 16, respectively.

Reduced socket volume and increase in bone density was detected in all subjects in a time-dependent manner. The radiographic images of the tooth socket in the axial, coronal, and sagittal views of each group are demonstrated in Figs. 3, 4 and 5, respectively. At the 3-month follow-up, new trabecular bone initiated from the surrounding walls and apex of the socket, and continuously grew toward the center of the socket, thus, the distribution of trabecular

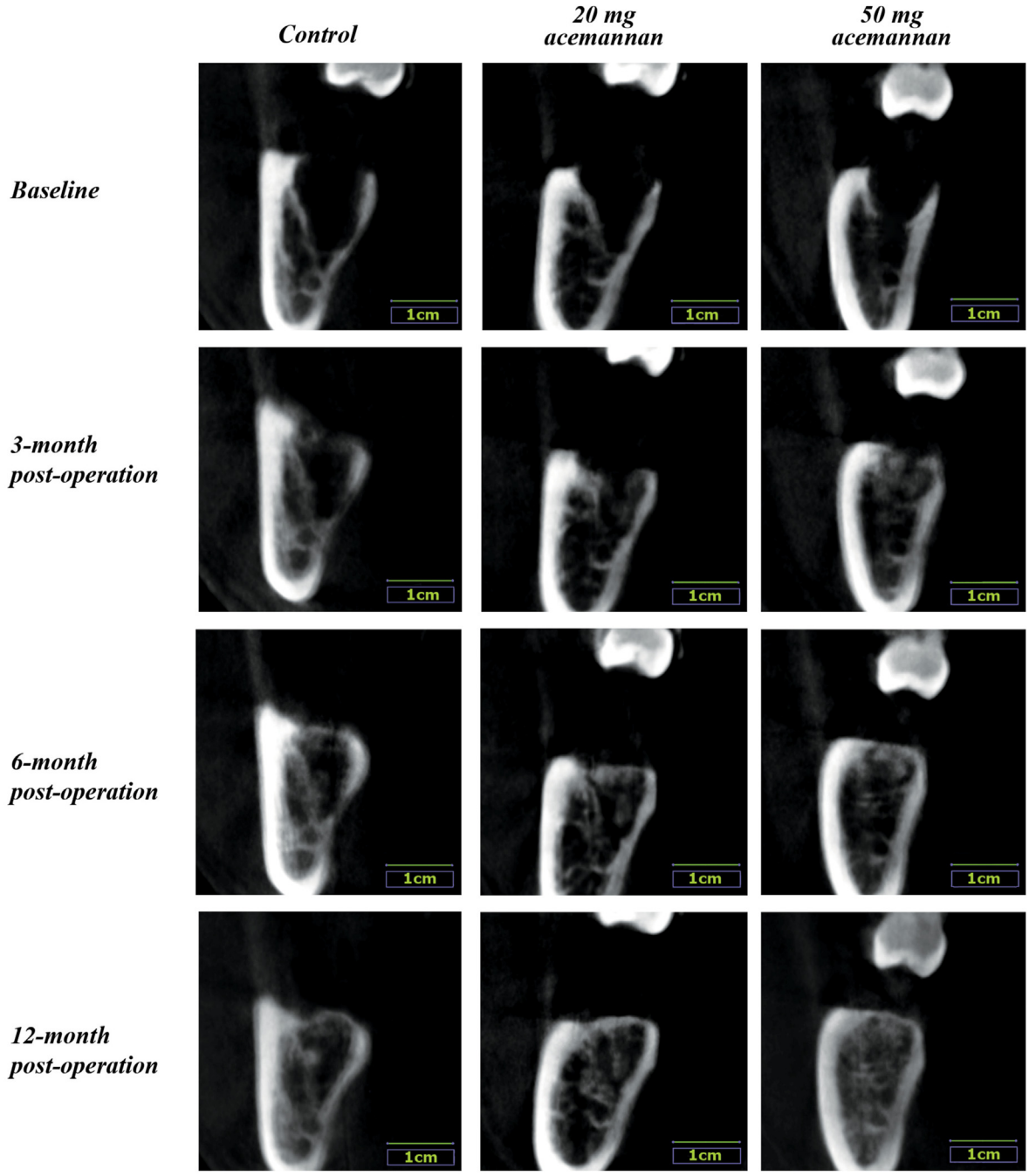

Figure 4 Representative radiographic images of the tooth socket: coronal views of the control, 20 mg acemannan, and 50 mg acemannan groups at baseline and three, six, and twelve months postoperatively. Images were generated using the OsiriX MD Program.

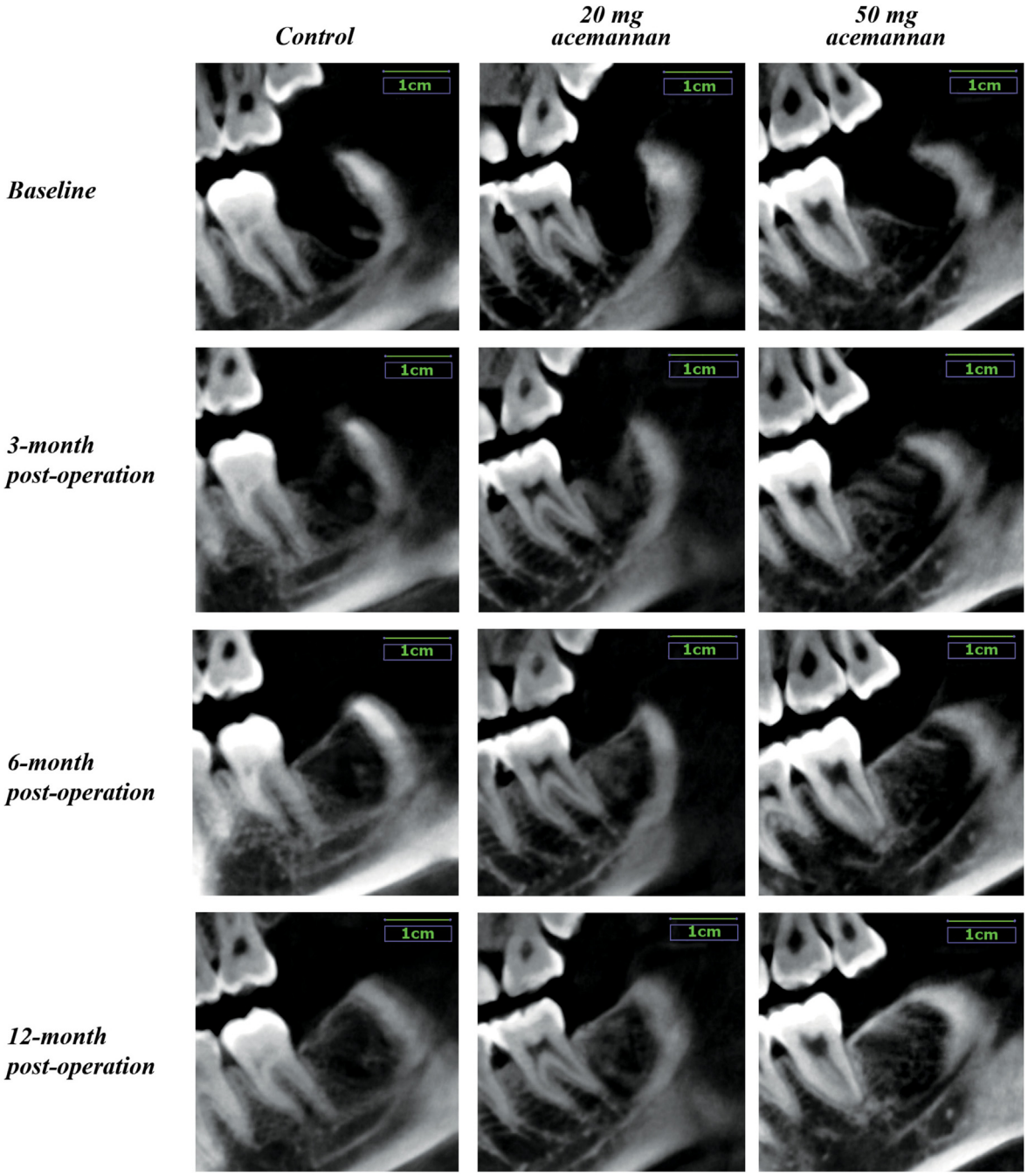

Figure 5 Representative radiographic images of the tooth socket: sagittal views of the control, 20 mg acemannan, and 50 mg acemannan groups at baseline and 3-, 6-, and 12-months postoperatively. Images were generated using the OsiriX MD Program.

density was restricted to the surrounding walls. Interconnecting honeycomb-shaped trabecular bone was observed in the socket at 6-months post-operation. The trabecular bone density was well distributed in the socket. The acemannan groups showed increased trabecular bone formation and interconnections compared with the control group. A thin layer of compact bone at the alveolar ridge that covered the socket was also found at this time point. From 6- to 12-months, the trabecular bone continuously filled the socket at a slower rate than the first 6 months. Thick compact bone at the alveolar ridge was also observed. However, no compact bone was detected in the center of the socket.

There were no significant differences in the socket volume at baseline between the three groups ($p > 0.05$; Fig. 6). The mean socket volume in each group significantly decreased during the follow-up period ($p < 0.05$). The largest and smallest reduction in the mean socket volume was found in the 50 mg acemannan and control groups, respectively, at each time point.

At 3-months postoperatively, the %ΔV values in the 20 and 50 mg acemannan groups were 1.3- and 1.41-fold,

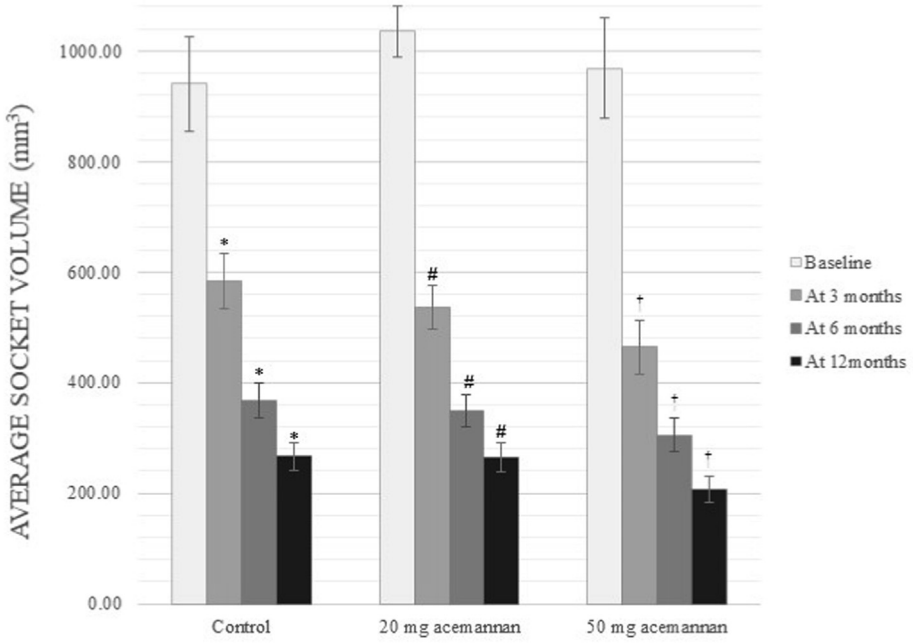

Figure 6 Acemannan reduced the average socket volume. The average socket volumes (mm^3) of the control (n = 14), 20 mg acemannan sponge (n = 13), and 50 mg acemannan sponge (n = 16) groups at baseline and 3-, 6-, and 12-months postoperatively are shown. Significant differences were found within the groups. Data are presented as means ± standard error. *, #, † indicate significantly different compared with its baseline for the control, 20 mg acemannan sponge, and 50 mg acemannan sponge groups, respectively; ($p < 0.05$).

respectively, higher compared with the control group ($p < 0.05$; Table 1). Significant differences in the %ΔV value were observed between the 50 mg acemannan and control groups at 6- (1.13-fold) and 12-months (1.1-fold) postoperatively ($p < 0.05$). No significant differences in the %ΔV between the 20 mg acemannan and control groups were observed at 6- or 12-months postoperatively ($p > 0.05$).

The null hypothesis was that the %ΔV*t* value at 12-months would not differ significantly between the acemannan and control groups. The mean %ΔV*t* values in the 50 mg acemannan (n = 16) and control (n = 14) groups were 77.64% ± 7.16% and 70.89% ± 9.6%, respectively. Therefore, 80% power with a type I error of 5% was achieved. The inter-observer and intra-observer reliability coefficients for data evaluation were 0.87 and 0.9, respectively.

Discussion

In our study, partially impacted mandibular third molars were used to evaluate the efficacy of acemannan for socket healing. Mandibular extraction sockets can be evaluated without interference from surrounding anatomical structures. This model represents a challenging osseous defect for investigation of the clinical efficacy of biomaterials on alveolar bone regeneration. Although removing bone to expose the impacted tooth causes a bony defect, only flap suturing is needed to cover the defect.[30] Therefore, no effects from extra materials involved in socket healing were observed.

To minimize the errors between groups, the number of mesioangular and vertical impactions were equally allocated. The study population consisted of non-smoking healthy patients 18–25 years old. All surgeries were performed by one surgeon, and the operation time was limited to 30 min. There were no significant differences in baseline socket volume between the groups.

CBCT has been recommended as a practical tool for clinical research investigation and evaluating teeth and jaw bone, as well as underlying anomalies and pathologies involving bone disease. The precision and accuracy of 3D data reveal distinct radiodensity differences between the dental alveolus and surrounding cortical bone and exclude superimposed anatomical structure interference; conversely, data from periapical and panoramic radiographs are limited.[31] We established high reproducibility of measurements using dedicated tools for CBCT analysis,[32] which minimized measurement errors and resulted in good inter-observer and intra-observer reliability.

Volumetric changes in bony defects have been used to evaluate the rate of bone healing.[33] Reduced volume suggests that the defect is filling with new bone. Therefore, the rate of bone healing is directly proportional to the percentage reduction in defect volume. In the present study, we measured the tooth socket volume by summing consecutive slice volumes from the coronal to apical parts of the extraction socket as previously described.[34] To standardize measurements and minimize errors, planes and reference points were set. The cementoenamel junction is more stable than the alveolar bone crest, which undergoes chronic resorption and remodeling during socket healing.[35] Our results showed that the socket volume was reduced in all groups during postoperative follow-up. Consequently, this parameter can be used as a radiographic indicator of

Table 1 Mean percentage reductions in socket volume at three **(A)**, six **(B)**, and twelve **(C)** months, postoperatively.

(A)

Groups	Mean (%)	S.D.	S.E.	
Control *(n=14)*	37.39	6.37	1.70	*
20 mg Acemannan *(n=13)*	48.67	9.04	2.51	*
50 mg Acemannan *(n=16)*	52.73	5.08	1.27	

(B)

Groups	Mean (%)	S.D.	S.E.	
Control *(n=14)*	59.53	11.61	3.10	
20 mg Acemannan *(n=13)*	66.64	6.29	1.75	*
50 mg Acemannan *(n=16)*	67.62	7.89	1.97	*

(C)

Groups	Mean (%)	S.D.	S.E.	
Control *(n=14)*	70.89	9.60	3.10	
20 mg Acemannan *(n=13)*	74.59	6.93	1.92	*
50 mg Acemannan *(n=16)*	77.76	7.16	1.79	*

*Significantly different compared with the control group, $p < 0.05$ (Dunnett's test).

bone healing. We also noticed no significant decrease in bone height on the distal aspect of the adjacent second molar in all groups at 12-months postoperatively that corresponded to the findings of Krausz et al. and Gröndahl & Lekholm regarding alveolar bone height after lower third molar extraction.[36,37]

In the present study, both the 20 and 50 mg acemannan sponges enhanced tooth socket healing. However, the osteoinductive activity of the 50 mg acemannan sponges was superior to that of the 20 mg acemannan sponges during the first 3 months and superior to that of the control group at all time-points examined. Therefore, we recommend 50 mg acemannan as the optimal concentration for tooth socket healing. This finding was consistent with our previous study in which 50 mg acemannan was shown to increase tooth socket radiodensity on periapical radiographs 3-months postoperatively.

Socket healing consists of two overlapping phases: the formative and remodeling phases. The formative phase can be further subdivided into the early fast and last moderate formative phases that end at 3- and 6-months postoperatively, respectively.[38] After that, the bone formation rate decreases. The remodeling phase is initiated at 6-months postoperatively and continues beyond 1-year postoperatively to complete socket healing. At 3-months postoperatively, our results showed the greatest reduction in socket volume in all groups, consistent with the findings of the classic study by Schropp, in which the bone healing rate in the tooth socket peaked within the first 3-months postoperatively.[39] Most clinicians recommend examinations at 3- and 6-months postoperatively to evaluate socket healing prior to second-stage implant placement. Our study has provided a useful option for pre-implant treatment, based on the concept that applying an osteogenic material would reduce the time needed prior to implant placement.[40]

Notably, the CBCT analysis demonstrated that newly formed bone grew more from the mesial, buccal, and lingual walls of the socket, compared with the apical area. A possible explanation is that periodontal ligament remnants were present. Most periodontal ligaments extend from the cementum of the root surface to the alveolar bone, but a small subset connect the apex to the alveolar bone.[41] Periodontal ligament tissue remains on the socket wall after tooth extraction and may contain cells that can

differentiate into osteoblasts, supporting bone formation.[42]

Although the mechanism by which acemannan induces bone healing remains unknown, our data revealed that the reduction in socket volume in the acemannan groups was greatest at 3-months postoperatively, which corresponds to the formative phase of bone healing. Acemannan might function as a bioactive molecule that enhances bone formation via upregulation of cell proliferation, osteoblast differentiation, mineralization, and secretion of bone morphogenetic protein, vascular endothelial growth factor, and type I collagen.[14,16]Alternatively, acemannan sponges could serve as scaffolds to enhance blood clot formation, support cell migration and attachment, and improve growth factor retention.[43] Immunomodulatory activity of acemannan via enhanced macrophage activity has been also reported.[21] Based on their monosaccharide composition (acetylated mannose, glucose, galactose) and 3D molecular structure, acemannan sponges can be used as resorbable and biocompatible materials in tooth sockets without disrupting the healing process.[44]

This study was limited in that we could not demonstrate the osteoinductive properties of acemannan via histological analysis because of ethical concerns.[19] However, the osteoinductive property of acemannan in bone healing has been histologically demonstrated in animal studies. In addition, the observation period was only a 12-month follow-up, which is relatively shorter than what was recommended in some studies to assess long-term treatment success.[45] In conclusion, acemannan is a biomaterial that may enhance tooth socket healing via its potential osteoinductive activity.

Declaration of competing interest

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

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