



Clinical and Radiographic Evaluation of Chitosan Particles in Treatment of Intrabony Periodontal Defects: A Clinical Trial

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ABSTRACT

Objectives: Intrabony defects are among the most important signs of progression of periodontal disease. Complete tissue regeneration is the ideal goal of periodontal treatment, and regenerative methods aim to achieve this goal. New studies have reported the positive efficacy of chitosan to enhance the recovery of bony defects. This study aimed to clinically and radiographically assess the efficacy of chitosan particles for treatment of intrabony periodontal defects.

Materials and Methods: In this clinical trial, 18 intrabony three-wall periodontal defects were randomly divided into three groups (n=6). The control group only received conventional flap surgery with a sulcular incision. In the second group, low molecular weight (100,000-300,000g/mol) chitosan was used in the three-wall intrabony defects during surgery while high molecular weight chitosan particles (600,000-800,000 g/mol) were used in the third group. The probing pocket depth (PPD), clinical attachment level (CAL) and radiographic defect depth (RDD) were measured at baseline and at 6 and 12 months later. Repeated measures ANOVA, and McNemar's test were used for statistical analysis.

Results: In both the control (P<0.001) and coarse chitosan (P=0.035) groups, a significant difference was noted in PPD before and after surgery. CAL was not significantly different among the three groups (P>0.05). No significant difference was noted on radiographs between the groups regarding the regenerated bone density.

Conclusion: Chitosan showed no positive efficacy for treatment of three-wall periodontal bone defects.

Keywords: Chitosan; Alveolar Bone Loss; Regeneration

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INTRODUCTION

Chronic periodontitis is known as an inflammatory disease of the tooth supporting tissues caused by certain microorganisms. This inflammatory process results in progressive degeneration of the periodontal ligament and alveolar bone. The objectives of periodontal treatment are to control and remove periodontal pathogens and regenerate

the lost periodontal tissue [1].

Intrabony defects and their management are among the interesting and fundamental topics of periodontics. Intrabony pockets are a major cause of development of vertical bone defects, which may have one, two, or three bony walls [1]. The treatment methods for intrabony defects are extensive, and initially include regeneration therapy, while creation of new

attachments and bone regeneration are the ultimate goal of periodontal treatments [1]. This goal led to the emergence of regenerative therapies for treatment of periodontal defects. For example, guided tissue regeneration (GTR) was developed, evolved and widely used in treatment of periodontal lesions [1]. Chitosan is a natural polysaccharide made from chitin. Chitin is found naturally in hard shells of shrimp and crabs [2]. The conversion of chitin to chitosan should be done in a way to ensure the production of high-quality and high-purity chitosan, free of contaminants such as proteins, endotoxins, and toxic metals. According to the acetylation level, chitosan may have variable physicochemical properties in terms of solubility and viscosity [3]. In addition to the degree of acetylation, the biological efficacy of chitosan depends on the acetylation pattern, degree of crystallinity, and its molecular weight [4].

Among the many biological properties of chitosan, its optimal biocompatibility, biodegradability, lack of allergens [4], non-toxicity [2], regenerative potential, film formation, and wettability [5] can be named. Chitin and chitosan have strong analgesic properties [6]. Chitosan activates the immune and inflammatory cells such as polymer-phosphonuclear, macrophages, fibroblasts, and endothelial cells [7], and accelerates wound healing [8]. Also, the antioxidant parameters are important for wound healing [9]. One of the main properties of this polymer is its antimicrobial activity, which is applied to a variety of microorganisms, including viruses [5], fungi, bacteria and algae [3].

Chitosan is a typical mucoadhesive polymer and an ideal carrier for oral mucoadhesive delivery [9]. The drug delivery routes include oral, nasal, parenteral, and transdermal administration. Transmucosal administration of drugs was also recently discussed [5].

Chitosan has antioxidant activity by removing the free radicals [10]. The formation of bacterial plaque and decalcification of enamel inhibit osteogenesis [3]. Blood coagulation, platelet aggregation and homeostasis are used in regeneration therapy [11]. Chitosan also has anticancer activity through induction of apoptosis, and can stop the cell cycle [12]. Its

other biomedical applications include manufacturing of surgical sutures, dental implants, artificial skin, bone remodeling, cornea contact lenses, and capsule materials [5]. According to Harikumer et al, [1] use of chitosan-collagen film as a membrane in GTR is effective for treatment of intrabony defects. According to Zhang et al, [13] chitosan membrane has the potential for use in GTR as a membrane damper in periodontal regeneration. Boynuegri et al. [14] used a chitosan gel for regeneration therapy. Despite the lack of a clinical difference, there was a radiographic increase in bone density. The aim of this study was to clinically and radiographically assess the efficacy of chitosan particles for treatment of intrabony periodontal defects.

MATERIALS AND METHODS

This study was approved by the ethical committee of Babol University of Medical Sciences (MUBABOL.REC.1395.146). It was also registered in the Iranian Registry of Clinical Trials (IRCT: 20100427003813N8), and written informed consent was obtained from all patients.

Study design and eligibility criteria:

In this randomized, double-blind (patient, clinician) clinical trial, 18 three-wall intrabony periodontal defects in 5 patients (2 males and 3 females) were randomly divided into three groups (n=6). The groups were matched in terms of sex and location of lesions. The plaque index of patients was below 20% according to the O'Leary's plaque index [15]. The exclusion criteria were systemic diseases, requiring prophylactic antibiotics to prevent bacterial endocarditis, intake of drugs interfering with periodontal improvement, smoking, contraindication for periodontal surgery, anomalies such as cervical enamel projections and concavity of the root surface, hemiseptal defects, caries and/or restorations in the adjacent tooth root, and poor patient cooperation after the initial periodontal treatment.

Clinical attachment level (CAL) was measured in the mesial and distal surfaces of the interproximal region with a Williams probe, and the mean value was recorded for each

tooth. Radiographic interpretation of the radiographs regarding bone changes was performed by a radiologist.

Randomization and blindness:

Five patients presenting to the Periodontology Department of Babol University of Medical Sciences with moderate chronic periodontitis and intrabony defects were included in this study. The defects were randomly assigned to 3 groups for treatment with flap surgery (control group), flap surgery with high molecular weight chitosan (group 2) and flap surgery with low molecular weight chitosan (group 3). The participants were randomly assigned to low molecular weight (100,000-300,000), high molecular weight (600,000-800,000) or control group in 1:1:1 ratio. Six defects were assigned to each group that included one defect between the maxillary canine and first premolar, three defects between the maxillary first and second premolars, and 2 defects between the maxillary or mandibular second premolar and first molar (Fig. 1). The sample size was determined to be six defects in each group based on the effect size of 1.6 [14] and 95% confidence interval with 80% study power. The in-charge clinician who performed the interventions was unaware of the assigned codes. Also, measurements of periodontal parameters were performed by another clinician who was blinded to the study arms. A maxillofacial radiologist blindly reported the osseous changes.

Study protocol:

Initial selection and diagnosis of patients with three-wall bone defects were routinely performed based on radiographs. Then, during the surgery and after flap elevation, the final diagnosis of three-wall intrabony defects was made, and the depth of lesions was measured by a Williams probe from the edge of the bone crest to the cemento-enamel junction. The clinical parameters were measured at the beginning of the study and right before the surgery around each tooth by a periodontist who was unaware of the therapeutic approach by using the Williams Probe (HU-Friedy; Chicago, IL, USA). The CAL and probing pocket depth (PPD) were

measured. The surgical procedure was performed following local anesthetic injection of 2% lidocaine and 1:80,000 epinephrine (Percocaine E; Daruo Pakhsh, Tehran, Iran). A sulcular incision was made with a #15 blade, and a full-thickness flap was elevated. The granulation tissue in the area was also removed. Then, scaling and root planing was performed without osteotomy. High molecular weight or low molecular weight chitosan (Acros Company, New Jersey, USA) particles were placed in three-wall bony defects in groups 2 and 3. For this purpose, chitosan was mixed with 0.9% sodium chloride and placed at the defect site (Fig. 2). Then, in each group, one collagen barrier membrane with crosslinking, thickness of 1-1.4 mm, and resorption time of approximately 45 days (CenoMembrane; Tissue Regeneration Corporation, Kish Free Zone, Iran) was placed over the bone defect, such that it covered the defect with 1 mm margin and the flap was returned to its original position and sutured with figure of 8 and simple sutures.



Fig. 1. Intrabony defect between the first premolar and second premolar, and between the second premolar and first molar



Fig. 2. Chitosan was mixed with 0.9% sodium chloride, placed at the defect site and covered with CenoMembrane barrier

All surgical procedures were performed by the same surgeon. Patients were requested to use 0.2% chlorhexidine mouthwash (Emad Pharmacy, Isfahan, Iran) twice daily for 2 weeks. Ibuprofen (Hakim Pharmacy, Tehran, Iran) was used 400 mg every 6 h if needed and amoxicillin (Hakim Pharmaceuticals, Tehran, Iran) was used 500 mg every 8 h for 7 days. The patients were recalled for suture removal after 2 weeks. Postoperative follow-up was done weekly for up to 8 weeks by professional cleaning of the teeth with prophylaxis cups. During the follow-up sessions, gingival plaque of patients was eliminated (if present). Oral hygiene instruction was repeated as needed at 6 and 12 months after surgery. The clinical parameters were measured again at these time points.

Clinical measurements:

The baseline clinical measurements were made with a periodontal probe. The recorded indices included the CAL as the distance between the cemento-enamel junction and the depth of pocket, and the PPD as the distance from the free gingival margin to the depth of pocket. CAL and PPD were recorded at the time of surgery (T0), at 6 months after surgery (T1), and at 12 months after surgery (T2).

Radiographic assessments:

The first radiograph was taken using a size 2 PSP digital sensor (Soredex, Helsinki, Finland) with the parallel technique. Bite registration was performed using acrylic resin (Duralay; Reliance, IL, USA). It was first recorded before the radiography to ensure the same occlusion in the next radiography. The next radiographs were taken at 6 and 12 months later with the same voltage, amperage, exposure time, and occlusion record. Images were recorded in DICOM format and processed with Digora for Windows version 2.5 (PCT; Soredex; Helsinki, Finland). The digital subtraction of before and after treatment images was done by Photoshop CS6 software (Adobe Systems, CA, USA). Serial digital images were superimposed. When two images are obtained from one object and the image intensities of corresponding pixels are subtracted, the difference between them will produce a uniform image. This technique is referred to as the digital subtraction radiography [16,17].

A reduction in density indicated bone resorption, and an increase in density indicated bone formation.

Statistical analysis:

Quantitative data were recorded as mean \pm standard deviation. To compare the mean values, repeated measures and one-way ANOVA parametric tests were used. For the qualitative variables, the McNemar test was applied. Data were analyzed with SPSS version 22, and $P < 0.05$ was considered statistically significant.

RESULTS

The patients were approximately the same in terms of age (35-41 years with a mean age of 39 years) and sex (two males and three females).

Radiographic findings:

Bony changes based on the treatment group and type of intervention are displayed in Table 1. In the control group, after 6 months, 3 defects did not change and 3 defects showed a higher density. After 12 months, no change was observed in the density of the affected areas as compared with 6 months.

In the low molecular weight chitosan group, 4 defects did not change after 6 months, and 2 defects showed bone formation. After 12 months, no change was observed in comparison with 6 months. In the high density chitosan group, 4 defects did not show any change after 6 months, and 3 of the 4 defects remained unchanged after 12 months. Two defects showed increased osteogenesis. Among the two defects that showed bone formation after 6 months, one still showed bone formation after 12 months, but the other one showed bone resorption. The majority of bone changes occurred during the first 6 months of surgery.

Clinical findings:

The mean PPD significantly decreased in all three groups at 6 and 12 months after surgery ($P < 0.05$). There was no significant difference in the pre-surgical PPD among the three groups ($P = 0.316$). Also, there was no significant difference in PPD at 6 ($P = 0.446$) and 12 months ($P = 0.133$) among the three groups. But in both the control and high molecular weight chitosan groups, there was a significant difference in PPD before and after surgery ($P < 0.001$ and $P = 0.035$; Table 2).

Table 1. Bone changes based on treatment groups, type of intervention and time of radiography

Groups	Time (m)	No change N(%)	Increase N(%)	P*
Control	6	3(50)	3(50)	0.508
	12	6(100)	0(0)	
LW chitosan	6	4(66.7)	2(33.3)	0.289
	12	6(100)	0(0)	
HW chitosan	6	4(66.7)	2(33.3)	1
	12	3(50)	2(33.3)	

m: months; LW: low-weight; HW: high-weight
*McNemar test

Table 2. Comparison of means and standard deviations of probing pocket depths (mm) among the study groups at different time intervals (months)

Groups	Baseline	After 6m	After 12m	P#
Control	0.81±3.16	0.44±2	0.37±1.91	<0.001
LW chitosan	0.31±3	1.2±2.58	0.37±1.91	0.095
HW chitosan	1.21±3.75	0.4±2.33	0.40±2.3	0.035
p\$	0.316	0.446	0.133	0.383*

m: months; LW: low-weight; HW: high-weight
#: repeated measures ANOVA; \$: one-way ANOVA; * the result of comparison of the interaction effect of time and treatment on pocket depth using repeated measures ANOVA

CAL improved in all groups. No significant difference was observed among the groups at baseline (P=0.402), at 6 months (P=0.730) or at 12 months after surgery (P=0.953). But in all groups, the difference in CAL was significant before and after surgery (P<0.05; Table 3).

Table 3. Comparison of means and standard deviations of clinical attachment level (mm) among the study groups at different time intervals (months)

Groups	Baseline	After 6m	After 12m	P#
Control	0.75±1.83	0.54±1	0.54±1	0.001
LW chitosan	0.37±1.41	0.64±1.2	0.49±0.91	0.022
HW chitosan	0.6±1.83	0.31±1	0.33±0.95	0.029
p\$	0.402	0.730	0.953	0.161*

m: months; LW: low-weight; HW: high-weight
#: repeated measures ANOVA; \$: one-way ANOVA; * the result of comparison of the interaction effect of time and treatment on pocket depth using repeated measures ANOVA

DISCUSSION

In this clinical trial, 5 patients including 2 males and 3 females were evaluated, and 18 three-wall bony defects received membranes or chitosan plus membranes. The results showed that 6 months after surgery, improvement was observed in all clinical parameters in all three groups. All treatment methods caused a decrease in PPD and an increase in CAL.

Contrary to very large bone defects evaluated in a previous study [1], in the present study, 3-wall intrabony periodontal defects were treated.

We assumed that the viable tissue surrounding the defects contains growth factors necessary to induce bone formation; thus, no osteogenic materials were used in combination with chitosan. But since the treated defects in this study were small to moderate intrabony defects, the amount of growth factors available at the site was probably not sufficient to target the susceptible cells with the help of chitosan scaffold, and therefore, it had no significant boosting effect on the regeneration process.

In a previous study on the effect of chitosan on bone regeneration, chitosan was used in combination with autogenous bone graft or other connective tissues, leading to the migration of viable bone cells to the site of injury [13]. Tissue regeneration is possible only when viable cells are present around the defect to affect the signaling molecules such as growth factors.

Harikumer et al. [1] conducted a study on the use of collagen-chitosan membranes for Infrabony periodontal defects. The patients were examined periodontally; 12 of them showed over 4 mm PPD along with 12 healthy controls. The difference in PPD between the patient group and the control group was statistically significant.

This study showed that the use of chitosan-collagen film as a membrane was effective in GTR of intrabony defects. Although our findings did not show a significant difference in bone changes between the treatment groups, the majority of bone formation occurred in the first 6 months. One factor

affecting the results of this study is the presence of collagen, which has a positive effect on the recovery of periodontal defects. In addition, chitosan was used in their study in the form of membrane while we used chitosan particles that are more likely to be absorbed compared with membrane.

In this study, digital subtraction radiography was used to evaluate the progression of regenerative therapy. Similarly, Boynuegri et al. [14] examined the effect of chitosan on periodontal regeneration by examining clinical and radiographic findings at 3 and 6 months after surgery. There was no clinically significant difference, but there was a significant difference in radiography between the use and no use of chitosan. The clinical results obtained were similar to ours. Duralay molding material was used in this study to standardize the process of radiography. However, it might have undergone some volumetric changes, probably causing errors in standardizing the position in the follow-up radiographs. This might have caused errors in the radiographic results in the present study. Spin-Neto et al. [18] evaluated the effect of biomaterials containing chitosan on bone defects in rats.

The rats were divided into 4 groups and they used high molecular weight chitosan, low molecular weight chitosan, low molecular weight chitosan-hydrochloride and high molecular weight chitosan hydrochloride. According to radiographic analysis, bone density increased in rats treated with biomaterials (both high and low molecular weight groups) at 15 and 60 days. The observations were similar to those in the control group. According to their study, biomaterials with chitosan base did not improve the bone density in rats. Their results were similar to ours.

CONCLUSION

The use of chitosan was effective in improving the PPD and CAL, but this effect was the same as that of the conventional treatment. Also, the size of chitosan particles did not affect the amount of bone formation in defects.

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CONFLICT OF INTEREST STATEMENT

None declared.

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