Using a Composite Graft of Natural 15% Chitosan Gel in the Management of Intrabony Defects: A Case Series

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Abstract

Introduction: Periodontitis is a chronic infection affecting the supporting tissues of the teeth which, if left untreated, eventually leads to tooth loss. Various grafting materials and barrier membranes have been used to repair periodontal intraosseous lesions. It has been shown that chitosan, a natural polymer, has potential to be used for periodontal tissue regeneration. Thus, it is possible that chitosan, in conjunction with bone grafting, might have good potential to be used for periodontal regeneration. The purpose of this case series was to evaluate the efficacy of chitosan, along with bovine porous bone mineral, in periodontal regeneration of intrabony defects.

Materials and Methods: Ten patients aged between 30 and 55 years with intrabony defects ≥3 mm and pocket probing depth (PPD) ≥5 mm were selected. All participants received chitosan gel (15% w/v) plus bovine porous bone mineral as a composite bone graft. Clinical and radiographic measurements were recorded at baseline, 3 months after healing and at 6 and 9 months. Significance was set at 0.05.

Results: After nine months, all the defects treated with this combination showed PPD reduction of 5.30 ± 0.822 mm, relative clinical attachment level (CAL) gain of 5.80 ± 0.499 mm, reduction in intrabony defect (IBD) depth of 3.00 ± 0.497 mm and defect resolution of 78.32 ± 5.80 %, all of which were statistically significant.

Conclusion: Within the limits of this study, this case series study suggests that chitosan gel, along with bovine porous bone mineral, has a promising role to play in periodontal regeneration.

Key words: Chitosan, Periodontal regeneration, Bovine porous bone mineral, Wound healing

Introduction

Methods for the management of periodontal disease have progressed considerably over the last two decades. However, in some cases inability to control the progression of periodontal disease results in osteolysis of the alveolar bone supporting the teeth leading to intrabony defects (Gupta, 2010; Krebs and Clem, 2006). The long-term prognosis of teeth may be adversely affected if the intrabony defects are not treated appropriately. Several approaches to facilitate regeneration in these osseous defects have been reported in the literature and include the use of an array of regenerative materials (Garrett, 1996; Laurell *et al.*, 1998), tissue regeneration methods using barrier membranes and a combination of both bone grafts and membranes (Joly *et al.,* 2002). Despite each regenerative material possessing its own unique regenerative potency, there are certain inherent biological and surgical limitations which restrain their widespread acceptance (Wagh, 2004; Suzuki *et al.,* 1989).

These concerns and limitations have stimulated considerable interest in the development of artificial materials, that are natural in origin, to be used as bone graft substitutes (Giannoudis *et al.,* 2005). Recently, the discovery of chitosan (a novel natural polymer), has been considered as a useful adjunct for regenerative periodontics (Klokkevold *et al.,* 1996) because of its biological properties such as biocompatibility, non-toxicity, anti-inflammatory, biodegradability and bio-adhesion (Senel *et al.,* 2000a; Senel *et al.,* 2000b; Ikinci *et al.,* 2002; Fakhry *et al.,* 2004; Aksungur *et al.,* 2004; Akncbay *et al.,* 2007).

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In dentistry, chitosan has been used in various forms such as mouthwashes and films (Vilasan *et al.,* 2013). Our research team has recently reported that chitosan can also be used as a local drug delivery system during non-surgical periodontal therapy (Babrawala *et al.,* 2016a).

Chitosan possesses several bioactive properties including antimicrobial properties, hemostatic activity, tissue regenerative capacity, osteoconductivity and induction of neovascularization that lead to accelerated bone growth (Park *et al.,* 2000; Wang, 2003; Chevrier *et al.,* 2007; Jayasuriya and Kibbe, 2010). Chitosan has also been evaluated for the treatment of bone lesions by incorporating it with platelet-derived growth factor-BB (PDGF-BB) (Park *et al.,* 2000), hydroxyapatite (Mukherjee *et al.,* 2003), GTR membranes (Shin *et al.,* 2005) and has shown high tissue compatibility with no evidence of inflammatory reaction. Boynuegri *et al.,* (2009) evaluated a combination of 1% chitosan gel with demineralized bone matrix (DBM) for the treatment of periodontal intraosseous defects and reported favorable bone fill. Subsequently, an *in vitro* study demonstrated that chitosan, when used in combination with bone grafts, at a concentration of 15% has a superior regenerative potential than other concentrations (Weir and Xu., 2010). Recently, we have reported that chitosan at a 15% concentration has the potential to induce regeneration of intraosseous defects (Babrawala *et al.,* 2016b). However, to date there have been no data published on the regenerative potential of chitosan at 15% concentration when combined with bone grafts. Hence, the present case series aimed to evaluate the efficacy of 15% chitosan gel when combined with bovine porous bone mineral for the treatment of intrabony defects.

Materials and methods

Source of data

A total of 10 patients; 7 males and 3 females, aged between 30 and 55 years old attending the outpatient section of Department of Periodontology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore were included in the study (study dates: September 2015 to August 2016). The study protocol was reviewed and approved by the institutional ethical committee and review board. The design, nature of the clinical trial and the potential risks if any were explained to the patients. A signed informed written consent for their participation was obtained from them.

Sample size determination

G power software was used for a priori computation of the sample size of our study by keeping effect size 0.4, α error 0.05. Using this data we arrived at a sample size of 10 with 80% statistical power.

Selection Criteria

Systemically healthy patients aged 30-55 years with the presence of localized PPD ≥5 mm, CAL ≥5 mm, 3-wall intrabony defect ≥3 mm deep (assessed by transgingival probing and to be confirmed after flap elevation) with the defect not extending to a root furcation area and no invasive periodontal therapy carried out in the past 6 months and associated tooth and neighboring teeth with ≤1 mm of tooth mobility were included in the study. Patients with unacceptable levels of oral hygiene (PI > 1.5; Silness and Loe., 1964), pregnant and lactating women, smokers, patients with suspected or known allergy to chitosan or on medications known to interfere with periodontal wound healing and immunocompromised patients were excluded from the study.

Phase 1 therapy (scaling and root planning) was performed and re-evaluation was carried out 8 weeks after completion of the initial therapy.

One examiner (IB) performed all the surgeries while another examiner (MLVP) performed all the clinical and radiographic measurements.

Inter-examiner and intra-examiner calibration

Prior to commencement of the study, inter-examiner and intra examiner calibration was achieved by examining 20 patients two times (24 hours apart). The examiners were considered as calibrated if the measurements recorded at baseline and 24 hours were analogous within 1 mm at the 95% level.

Clinical and Radiographic Measurements

Prior to surgery, pocket probing depth (PPD) and relative clinical attachment level (CAL) were recorded using a UNC-15 (Hu-Friedy, Chicago, IL, USA) manual probe from the apical extent of a customized acrylic stent that was grooved to the base of the defect to ensure reproducible placement of the probe for each successive measurement. All the radiographs were taken using a paralleling technique of radiovisiography pre-operatively at baseline and post-operatively at 3, 6 and 9 months. A calibrated measurement software (Digimizer, MedCalc Software BVBA, Belgium, version 4.0) was used for the radiographic measurements. The cementoenamel junction (CEJ), the crest of alveolar bone (AC) and the base of the defect (BD) were marked on the image. A line was drawn from CEJ to BD. The software then displayed the distance between these two points. The same procedure was then repeated to obtain the distance between CEJ and AC. Subtracting the two measurements; the depth of the osseous defect was obtained. The total bone fill was measured subtracting the depth of the osseous defect at 9 months from the baseline measurement (Mahajan and Kedige., 2015).

Formulation of 15% chitosan gel

After purification, chitosan was further prepared by dissolution-precipitation and dialysis, and reacetylation up to a degree of 50%. In order to produce gels at 37°C, 15% chitosan solution (chitosan + sterile distilled water at pH 7.2) was prepared followed by neutralization with sodium hydroxide. This process resulted in a small increase in viscosity and a very slow gelation capacity. Thermosetting properties were tested before and after lyophilization to produce a stable formulation. Addition of trehalose preserved the thermosetting properties. The 15% concentration of chitosan gel was prepared in as described by Weir and Xu (2010) at the Essence Biotech Research Laboratory (Kochi, Kerela, India).

Surgical procedure

After administration of local anesthesia with 2% lignocaine hydrochloride and epinephrine concentration of 1:80,000 (Lignox 2%, Indoco Remedies Ltd, Goa, India), full thickness mucoperiosteal flaps were raised on the buccal and lingual aspects of the involved sites. Thorough debridement was performed using area-specific curettes and ultrasonic scalers (*Figure 1A* and *1B*). Fifteen percent chitosan gel, in combination with bovine porous bone mineral (Bio-Oss™, Geistlich Biomaterials, Switzerland), was used as the bone regenerative material to fill the intrabony defect (*Figure 1C*). Thereafter, the flaps were sutured to their original position with 4-0 silk sutures (Mersilk, Ethicon, Johnson & Johnson, Himachal Pradesh, India) with a 16 mm reverse cutting, 3/8 circle, atraumatic swagged needle. Simple interrupted sutures were made to achieve primary closure (*Figure 1D*) followed by placement of periodontal dressing (Coe Pack, GC America Inc, IL, USA).

Post-operative wound management

Following surgery, 500 mg of amoxicillin every 6 hours for 5 days, 400 mg of ibuprofen every 8 hours and 0.2% chlorhexidine digluconate twice daily for 4 weeks post surgically were prescribed to the patient. Patients were reevaluated for pain, sensitivity and discomfort. Patients were recalled after 7 days for suture removal. Hard and soft tissue measurements were taken at 3, 6, and 9 months post-surgically (*Figure 2*).

Statistical analysis

The data collected were entered in Microsoft Excel and Statistical analyses were performed using the Statistical Package for Social Science (SPSS ver 10.5) software. Shapiro-Wilks test was used to test the normalcy of the data and the data were found to be normally distributed. Therefore, parametric statistical tests were used. To test differences in the defect level over time, Analysis of Variance (ANOVA) test was performed with significance set at the level of 5%.

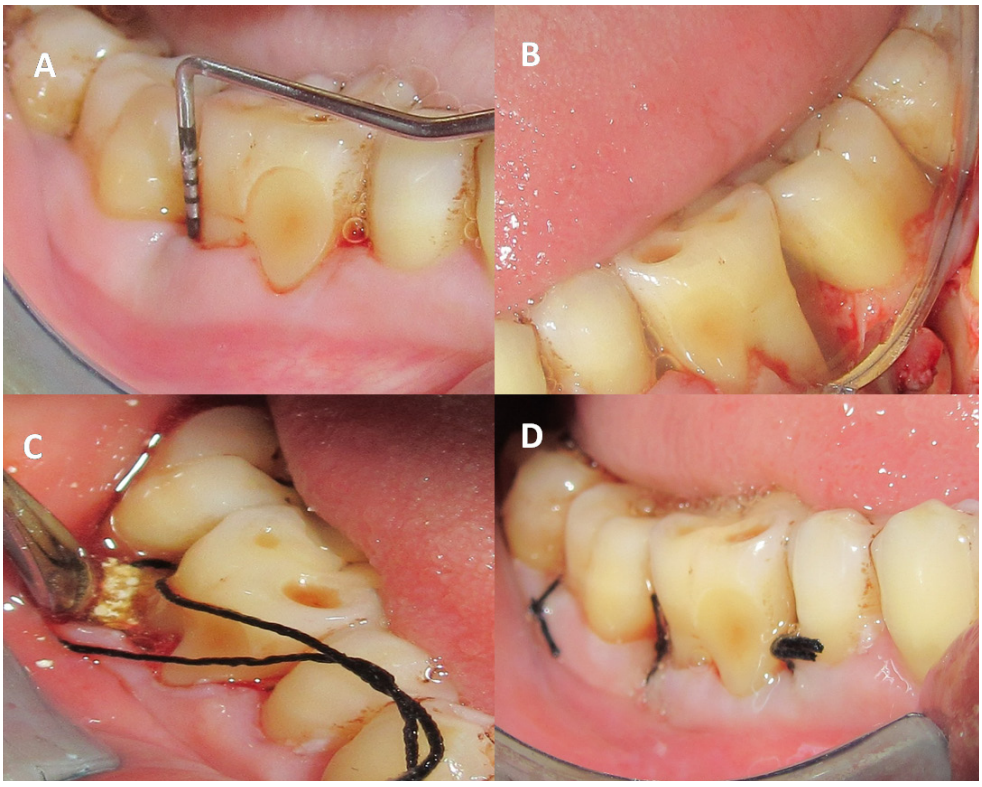

Figure 1. (A) Pre-operative measurement of pocket probing depth. (B) Raising full thickness mucoperiosteal flap followed by debridement. (C) 15% chitosan gel + bovine porous bone mineral packed in the defect. (D) Primary closure with simple interrupted sutures.

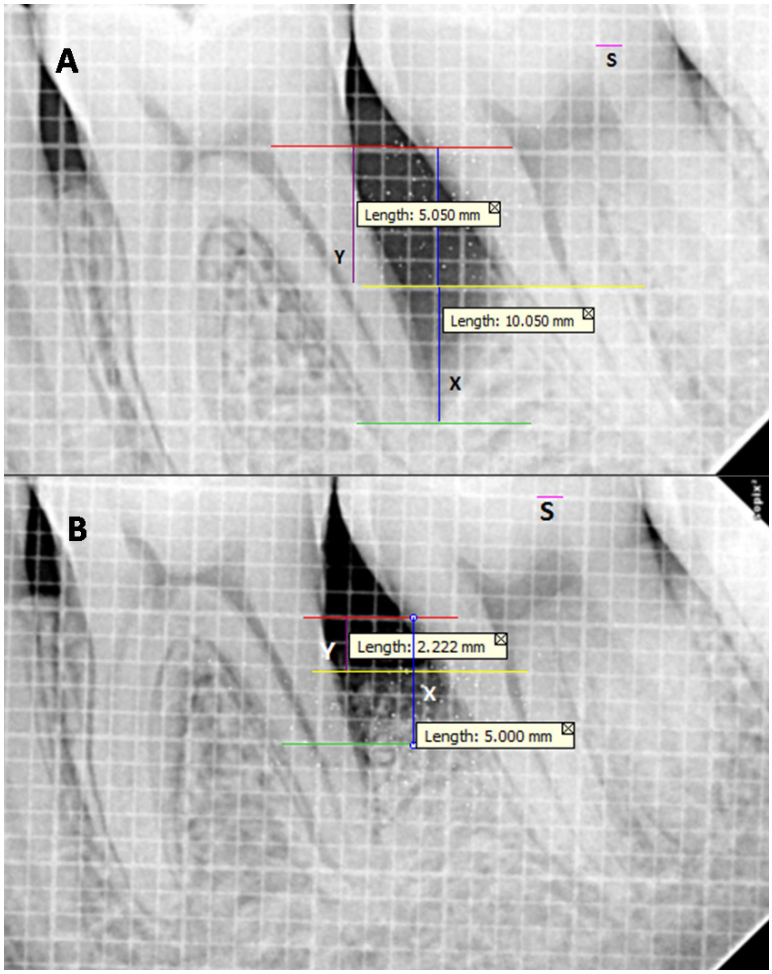

Figure 2. Radiographic analysis to determine the depth of the intrabony defect with 3 reference lines, i.e. red line represents the cementoenamel junction (CEJ) of the tooth; yellow line represents the level of alveolar crest (AC) and green line represents the base of the alveolar defect (BD) using a software known as Digimizer (ver 4.0). Standardization of the radiograph (S). X is the distance from CEJ to BD and Y is the distance from CEJ to AC. Depth of intrabony defect = X-Y. (A) Pre-operative radiograph. (B) 9 months post-operative radiograph.

Results

A total of 10 defects in 10 patients were evaluated. The treated sites were evaluated for clinical parameters at baseline, 3 months, 6 months and 9 months post-operatively using the ANOVA test. No patient dropped out during the study and uneventful healing was observed for all cases. Good oral hygiene was maintained by the patients during the study period.

Significant reduction in pocket probing depth (PPD) was observed from baseline (8.20 ± 1.39 mm) to 9 months (2.90 ± 0.56 mm). Similarly, significant gains in attachment were noted from baseline (8.90 ± 1.17 mm) to 9 months (3.10 ± 0.67 mm; *Table 1*).

Significant reduction in intrabony defect depth was observed from baseline to 3 months i.e., 1.30±0.06 mm (3.80 ± 0.91 mm - 2.50 ± 0.85 mm = 1.30±0.06 mm), 6 months i.e., 2.1±0.24 mm (3.80 ± 0.91 mm - 1.70 ± 0.67 mm= 2.1±0.24 mm) and 9 months i.e., (3.80 ± 0.91 mm - 0.80 ± 0.42 mm = 3.00± 0.49 mm; *Table 1*). Percentage of defect fill noted at 9 months was 78.32 ± 5.80 (*Table 2*). On an average, complete defect fill was noted in 50% of patients after 9 months.

Table 1. Intragroup comparative evaluation of pocket probing depth (PPD), clinical attachment level (CAL) and radiographic depth of intrabony defect (IBD) in millimeters (mm) at different visits

Parameter	Visit	Mean ± Std Deviation	*p* value
PPD	Baseline	8.20 ± 1.39	0.001*
	3 months	5.30 ± 0.67	
	6 months	4.10 ± 0.73	
	9 months	2.90 ± 0.56	
CAL	Baseline	8.90 ± 1.17	0.001*
	3 months	5.70 ± 0.82	
	6 months	4.20 ± 0.70	
	9 months	3.10 ± 0.67	
IBD	Baseline	3.80 ± 0.91	0.001*
	3 months	2.50 ± 0.85	
	6 months	1.70 ± 0.67	
	9 months	0.80 ± 0.42	

*p value ≤ 0.05 is statistically significant. ANOVA test

Discussion

This is the first study, to the best of our knowledge, in which a combination of chitosan gel at a concentration of 15% and bovine porous bone mineral has been evaluated for the treatment of periodontal bone defects. The outcome of the present study demonstrated that chitosan has good regenerative potential as both clinical and radiographic parameters improved significantly.

In the present study, no inflammatory reactions were noted, with total biological acceptance. Overall the post-operative healing was uneventful. To ensure standardization, all measurements were recorded by calibrated examiners and only 3- wall intrabony defects ≥3 mm affecting molars were considered. All the selected patients had a thick gingival biotype. The biotype was evaluated by placing the periodontal probe in the facial aspect of the gingival sulcus. It was categorized as thin if the outline of the underlying probe could be seen through the mucosa and thick if the probe could not be seen. Intrabony defects, when repaired with this combination, exhibited significant PPD reduction of 5.30 ± 0.822 mm, CAL gain of 5.80 ± 0.499 mm, reduction in IBD depth of 3.00 ± 0.497 mm and defect resolution of 78.32 ± 5.80 % at 9 months.

A previous study where a combination of chitosan gel at 1% concentration + demineralized bone matrix (DBM) (Boynuegri *et al.,* 2009) was used for intraosseous defects, reported a reduction in PPD of 2.60 ± 0.17 mm, gain in attachment of 1.80 ± 0.12 mm and the mean amount of intrabony defect depth observed at 6 months was 1.40 ± 0.08 mm. In comparison, the results of the current study were superior in all parameters even at 6 months. In addition, our results showed a similar trend with other studies where chitosan ws used in combination with collagen membranes or combined chitosan with hydroxyapatite and PDGF-BB, suggesting that chitosan improves clinical parameters and radiographic bone resolution (Park *et al.,* 2000; Mukherjee *et al.,* 2003; Shin *et al.,* 2005; Boynuegri *et al.,* 2009).

The favorable results noted in our current study can be assumed to be due to the favorable regenerative properties of chitosan. Primarily, chitosan may be considered as a very promising scaffold material in bone tissue engineering due to its ability to potentiate the differentiation of osteoprogenitor cells that may facilitate bone formation (Klokkevold *et al.,* 1996). Secondly, chitosan demonstrates structural similarities to the glycosaminoglycan hyaluronic acid which is found in extracellular matrices of many tissues. Hyaluronic acid is believed to facilitate the proliferation and migration of progenitor cells facilitating tissue regeneration (Adzick and Longaker, 1992). Thirdly, osteoblastic differentiation may be enhanced by chitosan which may interfere with the fibroblast function to inhibit bone formation and indirectly facilitate osteogenesis (Klokkevold *et al.,* 1996). The noteable advantage of chitosan is that its degradation product is a neutral to weak base sugar, as opposed to some graft materials that generate acidic degradation by products, evoking undesirable tissue reactions (Weir and Xu, 2010).

Despite the favorable results, the outcome of this study should be interpreted with caution as there are certain inherent limitations. Direct comparison between studies including the present one might not be reasonable due to large variation among them regarding patient and graft characteristics, defect morphology, surgical technique and wound management. The defects included in the present study were 3-wall defects which were deemed as having a reasonable potential for regeneration. Furthermore, it should be noted that the radiographs used in this study were not identical due to minor errors in film placement. Nevertheless, this technique of combining chitosan with bovine porous bone mineral should be explored further in defects with limited regenerative potential such as 1- and 2-wall defects. The present case series did not include a control group. Therefore, it is not known if the use of bovine porous bone mineral alone could also have resulted in acceptable defect fill in this study. Apart from small sample size and short follow-up, chitosan itself has some limitations despite the fact that it is a propitious material. This natural polysaccharide has poor solubility (Chen *et al.,* 2005) and lacks long-term stability. Since chitosan is a weak scaffold, the desired mechanical strength can be attained by using additional hydroxyapatite (Malafaya and Reis, 2009) or gelatin (Jiankang et al., 2009). With the analytical methods available today, histologic evaluation is needed for conclusive evidence of this therapeutic outcome.

It may also be worthwhile to evaluate chitosan with other regenerative materials. There is also a need for multi-centered long-term randomized clinical trials to be conducted in order to validate the outcome of this preliminary report. With the limited evidence available, it can be concluded that a combination of 15% chitosan gel and Bio-Oss™ that acts as a biological modifier might support and enhance periodontal regeneration. The present data indicates that the treatment of intrabony defects with 15% chitosan and bovine porous bone mineral resulted in considerable clinical and radiographic improvements.

Conflict of Interest

The authors reported no conflict of interest.

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