Efficacy of a Novel Zn-Substituted Monetite-Based Scaffold in the Treatment of Periodontal Osseous Defects

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Abstract

Purpose: The objective of this study was to evaluate the efficacy of a zinc-substituted nanostructured monetite-based scaffold (Sil-Oss®) in the treatment of periodontal intrabony osseous defects.

Methods: Thirty subjects participated in this study. Two sites in each subject were randomly assigned into each of the following experimental groups: Test group - open flap debridement (OFD) with Sil-Oss®; and control group - OFD with hydroxyapatite (HA) bone graft. Recorded clinical parameters included site-specific measures of plaque, probing pocket depth (PPD) and clinical attachment loss (CAL) at baseline, 3, 6 and 9 months. The evaluation of bone fill was performed by using digital subtraction technique and morphometric area analysis using two image processing software. Histological evaluation was done after 7 months by taking bone biopsy samples during crown lengthening procedures. Ten regions of interest (ROIs) per slide were visualized for mineralized tissue volume using an Olympus BX53® microscope at 40X magnification.

Results: Sil-Oss® showed a significantly greater bone fill compared to HA at 3 and 6 months. Sil-Oss®-treated defects also showed a marked increase in the percentage of tissue mineralization (25.38% vs 23.73%) compared to HA-treated defects. No significant differences were observed between the two groups for CAL and PPD at 6 months.

Conclusion: We conclude from this trial conducted over a period of 9 months that Sil-Oss® has the potential to function as a graft material for periodontal regeneration.

Key words: Periodontitis, bone loss, hydroxyapatites, regeneration, bone grafts

Introduction

The ultimate goal of periodontal therapy is to restore the structure, integrity and function of tissues that have been lost as a result of inflammatory periodontal disease (Philstrom *et al.*, 2005; Polson 1986). Bone grafts function as structural scaffolds and matrices for attachment and proliferation of anchorage-dependent osteoblasts (Meseguer-Olmo *et al.*, 2008; Chen *et al.*, 2010; Zohar *et al.*, 2005; Constantino *et al.*, 1994; Mankin *et al.*, 1996; Fujinami *et al.*, 2007; Stabholz *et al.,* 1977). Various synthetic alloplastic grafting materials have been introduced to overcome the limitations of autogenous bone grafts (Klokkevold *et al.*, 1999). The most commonly used alloplastic materials include porous hydroxyapatite, (Nery *et al.*, 1990), β-tricalcium phosphate (Oonishi *et al.*, 1997) and bioactive glasses (Albee, 1920). When placed in human periodontal defects, these have demonstrated osseous fill and probing depth reduction with limited evidence of connective tissue attachment (Klokkevold *et al.*, 1999; Nery *et al.*, 1990; Oonishi *et al.*, 1997; Albee, 1920).

Porous hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is the most extensively researched material in periodontal defects. It is biocompatible, resorbs slowly, and is hydrophilic with high compressive strength (Hench and West, 1996). Bioactive glasses are made of silicon dioxide (SiO_2), calcium oxide

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(CaO), sodium oxide (Na_2O), and phosphorus oxide (P_2O_5) and bond to bone through the development of a surface layer of carbonated hydroxyapatite. They have the ability to bond to both hard and soft tissues and exhibit osteoconductive and osteostimulatory effects. Although bone formation has been reported following the use of alloplastic materials, there is no evidence that these materials may stimulate the formation of new cementum with inserting collagen fibers (Schallhorn, 1970). Histological and histomorphometrical examinations confirm the excellent bone biocompatibility and osteoconductive properties of dicalcium phosphate cement. This material does not evoke any inflammatory response, but favors new bone formation comparable with autologous bone grafting. It has been used as a bio-absorbable barrier for guided tissue regeneration in periodontal defects to act as a stable scaffold for bone formation and provide adequate space for periodontal tissue regeneration. (Hench *et al.*, 1996; Saghaei *et al.*, 2011; Krejci *et al.*, 1987; Galgut *et al.*, 1992).

Sil-Oss® (AzureBio, Madrid, Spain) is a synthetic and inorganic bone graft material and is composed of a dicalcium phosphate, anhydrous (monetite), hydroxyapatite (HA), amorphous silica and trace amounts of zinc. It is manufactured by a proprietary process that avoids high temperatures (Hench and West, 1996; Padilla *et al.*, 2006). This results in a non-sintered material with a particle size between 0.25 - 0.4 mm, high specific surface area (65 m^2/g) and high interconnected porosity (60%) that favors a high degree of interaction with its biological surroundings (Padilla *et al.*, 2015; Padilla *et al.*, 2006). Sil-Oss® is resorbed both by a dual process of slow dissolution of its components and by active cellular remodeling. Sil-Oss® is an osteoconductive and osteostimulatory material and controlled dissolution of Sil-Oss® releases Ca, P, Si and Zn that stimulate regeneration processes while larger pores are formed allowing colonization of osteoclasts and osteoblasts involved in bone remodeling. It functions as a bioactive temporary scaffold maintaining the desired volume while it promotes bone regeneration and is replaced by new vascularized bone (Hench and West, 1996). The alloplastic property of the graft material avoids the risk of infection and adverse inflammatory reactions. Also, resorption of Sil-Oss® prevents possible adverse effects associated with long permanence of low resorbable materials. (AAP 2001; Chen *et al.*, 2010; Zohar *et al.*, 2005; Constantino *et al.*, 1994; Mankin *et al.*, 1996; Fujinami *et al.,* 2007; Stabholz *et al.*, 1977; Klokkevold *et al.,* 1999; Nery *et al.*, 1990; Oonishi *et al.*, 1997; Albee*,* 1920; Hench and West, 1996)

The primary objectives of this study were to 1) evaluate the efficacy of Sil-Oss® as a graft material in the treatment of intrabony defects on relevant clinical and radiographic periodontal parameters, and 2) if the clinical situation permits, to evaluate histologically the amount of mineralized tissue volume in the sites treated with Sil-Oss®.

Methods

Study design

The study was designed as a split-mouth, double-blind, randomized controlled clinical trial. Approval from the Institutional Review Board was obtained and the study is listed on http://www.clinicaltrials.gov (NCT02639572).

Sample size calculation

Sample size was calculated by considering this trial as a non-inferiority trial. A minimum sample size of 27 will be required when the minimum difference of mean bone fill levels before and after treatment is to be at least 1 mm^2 at $p = 0.05$ with expected variance of 0.8 for having ß = 0.1.

Source of data

Thirty subjects were selected from the outpatient section of the Department of Periodontics. Systemically healthy chronic periodontitis subjects within the age group of 30-55 years having at least 2 periodontal pockets ≥ 5 mm with at least 1 pocket in each quadrant showing radiographic evidence of vertical bone loss were included in the study. Patients who underwent periodontal therapy in the past 6 months and/or had used antibiotic drugs, antioxidants, and antibacterial mouthwashes or medicated toothpastes within 6 months of baseline, and smokers were excluded from the study. Assessment of suitability for bone graft was confirmed by transgingival probing to verify the presence of predominantly three wall interproximal defects ≥ 3 mm in depth after scaling and root planing.

Randomization and blinding

Randomization and blinding included computerized generation of the allocation sequence in random permuted blocks (block randomization) and blinded disbursement of medication (Saghaei*,* 2011). Allocation was performed by assigning the block of sites to study groups according to the specified sequence. Based on the sequence, the first operator selected two sites for each of the following experimental sites: the test site, in which a Sil-Oss® graft was placed, and the control site, which was treated by hydroxyapatite graft only. All the surgeries were performed by a designated operator for the sake of uniformity, whereas the relevant readings were recorded by the first operator who was blinded to the nature of the site. The blind was not broken until this clinical trial was completely finished.

Standardization of radiographs

Standard digital intraoral periapical radiographs were taken at baseline, 3 and 6 months by the paralleling/long-cone technique at preset parameters using a commercially available RVG (radiovisiography) system (Kodak RVG 5100® Digital Radiography System, Carestream Health, Rochester, NY, USA). After the imaging plate was placed in the film holder for paralleling technique (XCP Kits for Digital Sensors®, BlueDent, Chennai, India), silicon impression material (Elite HD + Regular Body Normal Set®, Zhermack, Badia Polesine, Italy) was added around the biting surface and allowed to set. This arrangement ensured standardized alignment of the aiming device and the holder ensuring correct positioning of the collimator in subsequent radiographs.

Study protocol

Prior to the surgical phase, all subjects received standard periodontal therapy including oral hygiene instructions, occlusal adjustment, scaling and root planing (SRP). Thereafter, patients underwent a stringent maintenance schedule at 1-month intervals. Decision to perform periodontal surgery was made based on re-evaluation performed at 4 months after initial therapy. Sites not selected for the trial received appropriate surgical therapy. After the interdental areas were probed buccally and lingually/palatally, the site was considered for study if the average probing pocket depth (PPD) was ≥ 5 mm. All baseline (on the day of surgery) parameters were recorded before the surgical procedure. Probing pocket depth, clinical attachment level (CAL) and site-specific plaque scores were recorded at baseline, 3, 6 and 9 months.

Surgical procedure

At the start of the surgical procedure, the patients were asked to rinse with 0.2 % chlorhexidine for 1 min. The area subjected to surgery was anesthetized by nerve block/infiltration depending on the surgical site using local anesthesia. Crevicular incisions were made and the flaps were elevated by means of blunt dissection with the help of a periosteal elevator. The osseous defect was debrided of granulation tissue and the root surface was planed to remove plaque and calculus, until a smooth hard consistency was found. The defect's architecture was confirmed by direct observation and classified based on number of bony walls present. In patients selected for the test group, in addition to open flap debridement (OFD), Sil-Oss® bone replacement graft was utilized to fill the defects to the most coronal level of the osseous walls. The required amount of composite alloplast (Sil-Oss®) was dispensed into a sterile dappen dish, mixed with the patient's own blood and carried to the defect site with an amalgam carrier. The mucoperiosteal flaps were repositioned and secured in place using interrupted sutures. The surgical procedure in control sites included OFD followed by placement of hydroxyapatite graft (G-graft®, Saharanpur, UP, India). The surgical area was protected and covered using a periodontal dressing.

Radiographic assessment

A single operator evaluated the bone fill by using digital subtraction technique and morphometric area analysis using specific tools in two image processing software applications according to a previously described method (Yellarthi *et al.,* 2014).

Digital subtraction technique and morphometric analysis

The radiographs obtained at 3 and 6 months were subtracted from the radiograph taken at the baseline by using commercially available image processing software (Adobe Photoshop® 6.0, Adobe Systems, San Jose, USA). To reduce brightness and contrast variations, both images were adjusted based on the levels and curves in the software. Before digital subtraction, both radiographs were moved in appropriate directions as needed to reduce geometric distortion *(Figure 1).* These images were then superimposed and subtracted by selecting the Image > Calculation > Exclusion > New channel tools. The excluded interdental layer was outlined by using the polygonal lasso tool and the layer was copied and saved as a separate joint photographic expert group (jpeg) document at low compression. After digital subtraction, the digitized and excluded interdental layer was transferred to open source software for area calculation (ImageJ®, Research Services Branch, NIH, Bethesda, Maryland, USA) for area calculation. The layer was converted into a grayscale image, and the measurement scale was set to account for any magnification/reduction of the radiograph because of RVG. The area of the layer was calculated (in mm^2) by initially enclosing the entire area with the rectangular selection tool and then by using the Analyze > Analyze particles tool *(Figure 2).*

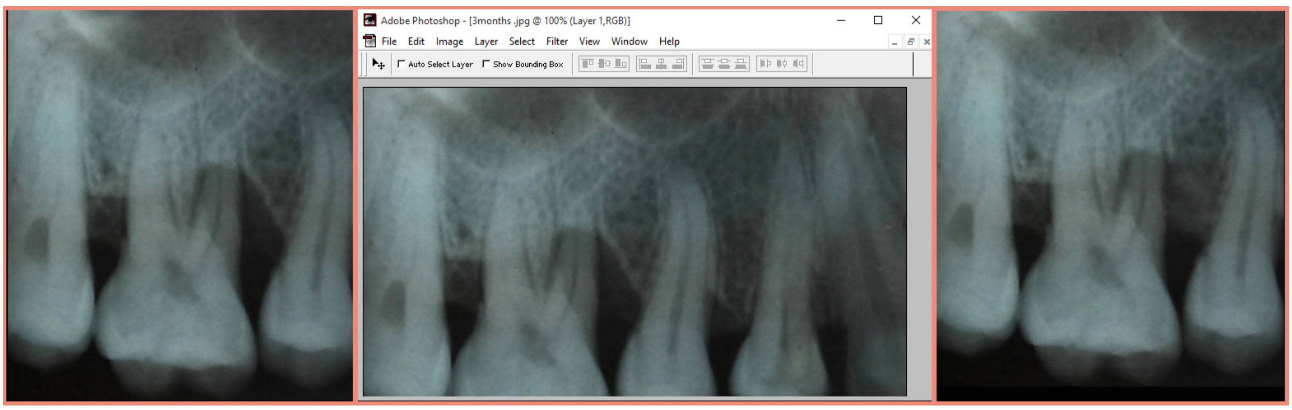

Figure 1. Sil-Oss® group: Pre- (left) and post-treatment (right) radiographs were superimposed (middle) using Adobe Photoshop®.

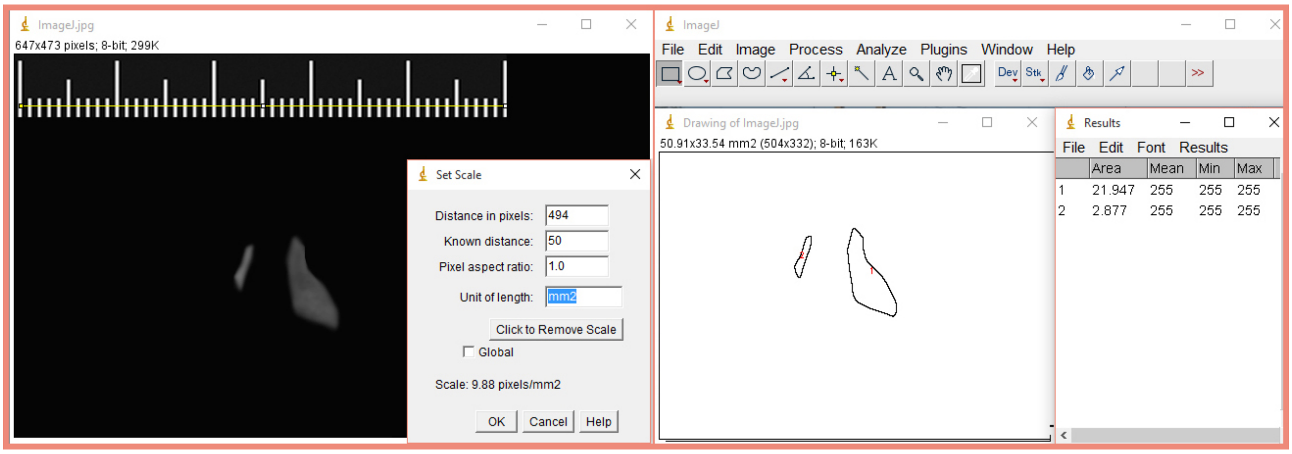

Figure 2. Morphometric area analysis was performed after digital subtraction and was calculated (in mm²) after converting the layer into a gray scale image in ImageJ.

Histomorphometric analysis

Bone biopsy specimens were obtained during crown lengthening procedures between 7.5 to 9 months from three sites each in both groups. Briefly, the specimens were immersed in 4% buffered formalin and were subsequently dehydrated in an ascending series of ethyl alcohol. The specimens were then stained using hematoxylin-eosin for light microscopy analysis. Eleven and 12 slides were prepared from Sil-Oss® and HA groups respectively. Ten regions of interest (ROIs) per slide were visualized for mineralized tissue volume by using an Olympus BX 53 microscope at 40X magnification. Before evaluation of bone sections in ImageJ, black and white image masks were created using Adobe Photoshop® according to a technique described by Egan *et al.,* 2012 *(Figure 3).*

Calibrating ImageJ

To calibrate ImageJ, a scale bar was placed on one image for each magnification. The file was opened with an image containing a scale bar inserted by the microscope or camera software that acquired the image. The length measured for the scale bar was entered as distance in pixels. The length of the scale bar as labelled by the microscope is entered as known distance and subsequent analysis was measured on this scale.

Quantifying mineralized tissue volume in ImageJ

The bone volume mask file was opened and the total area was selected by Edit > Selection > Select All and clicking Analyze > Measure. The "wand tool" and shift key were used to select the black areas. Selecting Analyze > Measure quantifies the mineralized tissue. The mineralized tissue volume was expressed as (mineralized tissue/total area)*100 *(Figure 4).*

Statistical analysis

Site-specific intragroup comparison between various groups was performed using ANOVA followed by multiple comparisons using Bonferroni correction. One-way ANOVA followed by the post hoc test was used for intragroup and intergroup comparisons. A p-value of < 0.05 was considered statistically significant and a p-value of < 0.001 was considered as highly significant.

Results

Thirty subjects examined from March to December, 2014 (mean age: 40.27 ± 9.66) were included in the initial phase of the study. Of the 30, three subjects were excluded due to sampling errors, thus limiting the final sample size to 27 subjects.

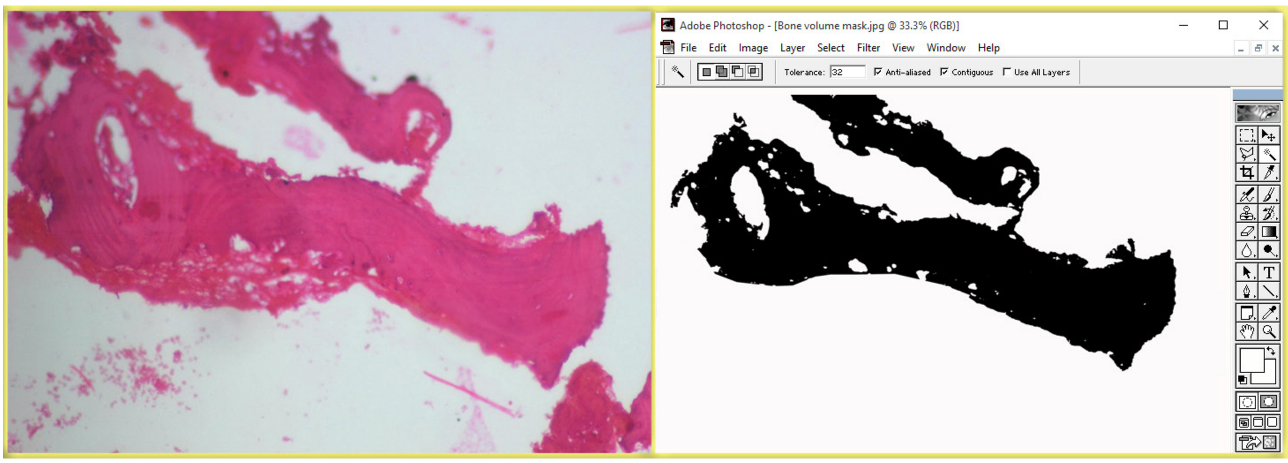

Figure 3. Before evaluation of bone sections in ImageJ, black and white image masks were created using Adobe Photoshop®.

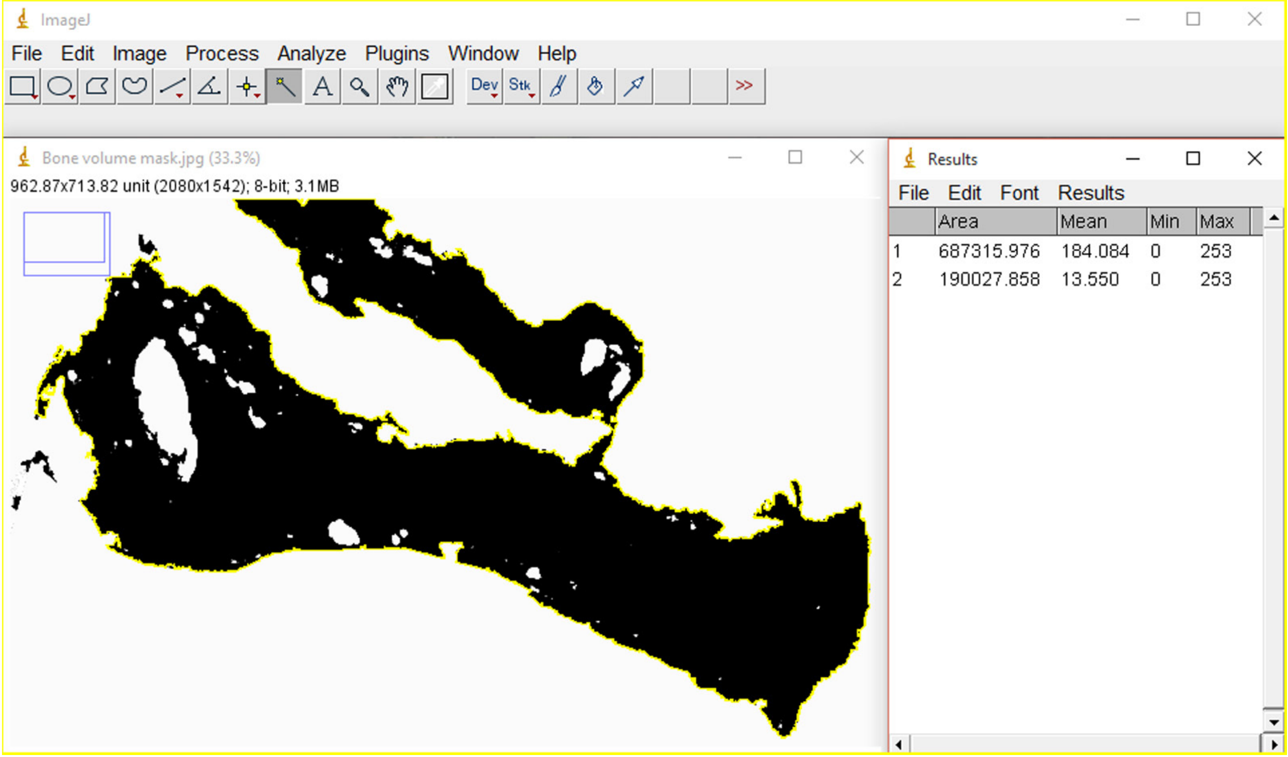

Figure 4. The mineralized tissue volume was expressed as (mineralized tissue/total area)*100. In this case it was (190027.858/687315.976)*100 = 27.64%.

Intra-group comparisons

Probing pocket depth

The mean probing pocket depths (in mm) in the control group were 7.58 ± 1.47, 3.96 ± 0.79, 3.09 ± 0.47, and 2.90 ± 0.53, and in the test group were 7.56 ± 1.38, 4.86 ± 0.89, 3.33 ± 0.75, and 2.93 ± 0.58 at baseline, and at the end of 3, 6 and 9 months respectively. The intra-group reduction in pocket depth when compared from baseline to 3, 6 and 9 months was statistically highly significant in both treatment groups ($p < 0.001$).

Clinical attachment loss

The mean clinical attachment loss (in mm) in the control group was 5.93 ± 1.22, 3.63 ± 0.88, 3.16 ± 0.79, and 3.06 ± 0.73, and in the test group were 5.90 ± 1.24, 3.46 ± 0.93, 3.00 ± 0.90, 2.93 ± 0.98 at baseline, 3, 6 and 9 months respectively. This intra-group reduction in clinical attachment loss when compared from baseline to the end of 3, 6 and 9 months was statistically highly significant in both treatment groups ($p < 0.001$).

Plaque index

The mean site-specific plaque index scores in the control group were 1.55 ± 0.05, 1.22 ± 0.04, 0.89 ± 0.06, and 0.89 ± 0.06, and in the test group were 1.56 ± 0.39, 0.32 ± 0.05, 0.30 ± 0.05, and 0.39 ± 0.07 at baseline and at the end of 3, 6 and 9 months, respectively. This decrease in plaque scores when compared from baseline to 3, 6 and 9 months was statistically highly significant ($p < 0.001$) in both treatment groups.

Bone fill

The change in mean bone fill (in mm^2) when compared from baseline to 3 months and 6 months in the control group was 9.82 ± 1.77 and 11.80 ± 1.91, and in the test group was 10.98 ± 1.87 and 13.40 ± 2.39, respectively. This intra-group gain in bone fill when compared from baseline to 3 months and from baseline to 6 months was statistically highly significant in both treatment groups ($p < 0.001$).

Intergroup comparisons

Bone fill and mineralized tissue volumes

In radiographic analysis, Sil-Oss® showed a significantly higher bone fill compared to HA at 3 and 6 months ($p < 0.05$; *Table 1*). Sil-Oss® also showed a marked increase in the percentage of tissue mineralization (25.38 ± 2.94% vs 23.73 ± 2.75% in sites treated with HA). This difference, however, was not statistically significant (*Table 1*).

PPD, CAL and PI

No significant differences were observed between the two groups for CAL at different time intervals. At baseline, there was no significant difference in PPD between the groups. At 3 months, however, the difference was highly significant ($p < 0.001$), with HA showing lower probing depths than Sil-Oss® at 3 months. This difference was not significant at 6 and 9 months. The plaque index was significantly different only at the 9th month (*Table 2*).

Table 1. Intergroup comparison of bone fill and mineralized tissue volume at different time-based intervals.

	Group	Mean ± SD	*t*-value	*p*-value
Bone fill (mm^2)				
Baseline - 3 months	Sil-Oss®	10.98 ± 1.87	2.606	0.012*
(n = 27)	HA	9.77 ± 1.77		
Baseline - 6 months	Sil-Oss®	13.40 ± 2.39	3.019	0.004*
(n = 27)	HA	11.72 ± 1.94		
Mineralized tissue volume (%)				
(n = 78 ROI)	Sil-Oss®	25.38 ± 2.94	1.382	0.657†
(n = 96 ROI)	HA	23.73 ± 2.75		

*$p \leq 0.05$; †not significant; ROI, regions of interest

Table 2. Intergroup comparison of clinical attachment level and probing pocket depth at different time-based intervals using ANOVA

n = 27	Group	Mean ± SD	*t*-value	*p*-value
Clinical attachment level (mm)				
Baseline	Sil-Oss®	5.90 ± 1.24	-0.0312	0.756†
	HA	6.00 ± 1.26		
3 months	Sil-Oss®	3.46 ± 0.93	-0.767	0.446†
	HA	3.64 ± 0.87		
6 months	Sil-Oss®	3.00 ± 0.90	-0.743	0.461†
	HA	3.16 ± 0.77		
9 months	Sil-Oss®	2.93 ± 0.98	-0.731	0.468†
	HA	3.09 ± 0.74		
Probing pocket depth (mm)				
Baseline	Sil-Oss®	7.56 ± 1.38	-0.038	0.970
	HA	7.58 ± 1.47		
3 months	Sil-Oss®	4.86 ± 0.89	4.130	0.000**
	HA	3.96 ± 0.79		
6 months	Sil-Oss®	3.33 ± 0.75	1.457	0.152†
	HA	3.09 ± 0.47		
9 months	Sil-Oss®	2.93 ± 0.58	0.209	0.835†
	HA	2.90 ± 0.53		

†Not significant; **highly significant ($p \leq 0.001$); HA, hydroxyapatite

Discussion

Meta-analysis of various controlled clinical studies (Krejci *et al.*, 1987; Galgut *et al.*, 1992; Yukna *et al.*, 1998) have reported that various bone grafts result in significantly greater attachment gain with respect to conventional open flap debridement alone (Trombelli *et al.,* 2002). This study compared the response of periodontal osseous defects treated by a novel composite alloplast. This graft material was biocompatible and well tolerated, as evidenced by the absence of inflammation or infection, and uneventful healing. Sil-Oss®, a novel biomaterial that combines Zn-substituted monetite (57 wt%), hydroxyapatite (25 wt%), amorphous calcium phosphate (11 wt%) and hydrated silica gel (7 wt%), had a marked impact on periodontal clinical parameters, including probing pocket depth, clinical attachment loss and bone fill, as compared to control sites. This improvement can be attributed to various bioactive properties of the constituent materials, including the ability to form new bone (Schallhorn 1970; Padilla *et al.*, 2015; Padilla *et al.*, 2006). The particle size of 0.25 - 0.4 mm results in a pore size greater than 100 μm, allowing vascularization and new bone formation (AAP 2001; Klokkevold *et al.,* 1999; Nery *et al.*, 1990).

The results of the present study demonstrate that both the grafts were effective in reducing PPD and improving CAL, although the mean differences in PPD between the groups were statistically significant in favor of the HA group. This is in agreement with a previous study which reported improvement in periodontal parameters when pure HA and HA-based composite graft materials were compared with OFD alone (Reynolds *et al.*, 2003). Composite grafts of HA with additive materials have shown beneficial effects in periodontal regeneration. Monetite is a dicalcium phosphate bioceramic;

Nery *et al*., (1992) found that HA/dicalcium phosphate bioceramic combination appears to demonstrate greater gain in attachment level and bone regeneration in the treatment of periodontal osseous defects. In composition the material used in their study is closer to the material used in the present study.

A statistically significant increase in bone fill was seen in the Sil-Oss® group when compared to HA. This increased bone fill can be attributed to soft and hard tissue improvements following resolution of inflammation and to the osteogenic potential of the bone graft material used in the study. The presence of hydrated silica gel can impart beneficial properties and the results are in agreement with previous studies of Froum *et al.* (1998) Lovelace *et al.* (1998) and Mengel *et al.* (2003) who had reported 4.26 mm, 3.07 mm and 3.8 mm reductions in PPD respectively over a period of 6 months in sites treated with bioactive glass. This is also in agreement with a study by Park *et al.* (2001) who reported a mean bone fill of 2.8 mm in intrabony defects treated with pure bioactive glass; a significant radiographic osseous defect fill of 2.42 mm when compared to controls was observed with the Sil-Oss® group in the present study after 6 months.

The Sil-Oss® group also showed significantly lower plaque scores at the end of the study period. However, a minimum amount of plaque does not interfere with the regenerative process and all the patients maintained their oral hygiene properly throughout the study periods.

In histological evaluation, Sil-Oss® showed a higher but non-significant increase in mineralized tissue volume over HA alone. This is in agreement with the study by Kruse *et al*., (2011) who found increased percentage of newly formed bone in sites treated with HA/ silicate glass than HA alone (21% vs 17 %) at 4 weeks. However, the percentage of mineralized tissue volume in this study was lower when compared to the study done by Scarano and colleagues (2006) who reported 32% of newly formed bone using HA at 6 months, and to that of a study by Choi *et al.* (2011) who found 67% of new bone formation at 3 months. Bone formation is inherently dependent on subjects' healing patterns, and this variable may have contributed to the lesser values observed in this study (Polson, 19986 Kruse *et al.,* 2011; Scarano *et al*., 2006).

In view of the present findings, both OFD + HA and OFD + Sil-Oss® particles were effective in the regeneration of infrabony periodontal defects. However, pertinent histomorphometric and radiologic analyses seems to indicate that Sil-Oss® can lead to better results compared to HA alone. Equally important was the fact that the newly developed composite graft material did not cause any biological complications.

From this trial conducted over a period of 9 months, Sil-Oss® has shown the potential to function as a graft material for periodontal regeneration. Further clinical and histological studies are required in order to evaluate the efficacy of this graft material in the treatment of periodontal intrabony defects.

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