procedures were used [14]. A xenograft is tissue transported between genetically dissimilar members of different species. It is osteo-conductive, biocompatible and structurally similar to human bone. There are two sources of xenografts used for bone replacement in periodontics: bovine bone and natural coral. A purported advantage of this product as a bone substitute is that it is natural in that it can provide structural components similar to that of human bone, improving its osteoconductive capability compared to that of synthetically derived mineral Aichelmann-Reidy and Yukna [15].

Bovine derived HA bone substitutes increase the available surface area that can act as an osteoconductive scaffold because of their porosity. This HA mineral content is comparable to that of bone, allowing it to become well vascularized and integrate with new host bone (Thaller., *et al*. 1994; Chen., et al. 1996) [16,17]. A statistically significant gain of clinical attachment and reduction in probing depth has been demonstrated when bovine bone was compared to a non-graft control for the treatment of human vertical osseous defects. When compared to demineralized freeze-dried bone allograft (DFDBA), similar amounts of probing depth reduction, clinical attachment level gain, bone fill and defect resolution were obtained Richardson [18].

Hence in this present study an effort was made to evaluate the effect of TI-OSS® bovine xenograft in treatment of intrabony defects with single flap approach in smokers and non-smokers. The present study showed significant reduction in the means of Plaque index (PI), sulcus bleeding index (SBI), probing depth (PD) and gain of Clinical attachment level (CAL) for both the smoker sites and

Non-smoker sites after 6 months, but the mean reduction of PI, CAL and SBI between the sites showed no statistical significant association after 6 months. This denotes the application of Bovine Xenograft did not reveal any additional benefit in the reduction of PI, CAL and SBI. These results are concomitant the study of Trombelli [19]. This should be because of the regular and frequent recall visits in which the patients underwent regular supra-gingival scaling and motivation for oral hygiene practice, which further minimized plaque accumulation. This implied that the participants of the study exercised good oral home care.

Periodontal pocket is deliberated as the pathognomonic sign of periodontal disease and reduction in probing pocket depth is one of the requisites for successful periodontal therapy. Probing depth reduction in the present study was found to be significant from baseline to six months in the smokers' sites and Non-smoker sites.

Indicating that bovine xenograft has an added benefit on probing depth, this is similar to findings of a study of Yilmaz [20]. Ti-oss® is prepared from 100% cancellous bone without any cortical portion. Innovative pulverizing technique allows multi-porous structure, maximizing blood vessel ingrowth, Pre-Hydroxy Apatite structure, octacalcium phosphate crystal is found on the surface of Ti-oss®, resulting in fast bone formation. In the present study bone gain was 30.73% in smoker group sites and 53.64% in Non-smoker group sites ($p = 0.002$). This attributed the placement of bovine xenograft with single flap approach which provides a significant postoperative protection from exfoliation of the graft from the surgical site. The obtained results are in accordance with study conducted by Tombelli [19].

The purpose of developing these new techniques is to increase predictability, reduce patient discomfort, minimize the number of surgical sites and satisfy aesthetic demands of the treatment. When comparing early wound healing index at 2 weeks after the surgery, smokers showed a significantly lower number of sites with optimal wound healing and a higher number of sites with incomplete flap closure when compared to nonsmokers (0% vs 45.5% and 45.5% vs 18.2%, respectively), indicating a detrimental effect of smoking on early wound healing. This finding can be explained, at least in part by the alterations of the gingival vascular apparatus induced by the smoke [21,22] which in turn may have impaired blood perfusion. Blood perfusion was demonstrated to be a key determinant of the early healing of muco-periosteal flaps, and its impairment is frequently associated with wound dehiscence [23].

Treatment with bovine xenograft using single flap approach at defects resulted in substantial clinical attachment gain, with no significant intergroup difference. The magnitude of treatment effect is consistent with previous clinical trials investigating the use of bovine xenograft in the treatment of intraosseous defects [24]. In standardized periodontal defects experimentally created in rat molars, Azuma., *et al.* [25] demonstrated that the elevation of a single flap. A lower number of neutrophils, more rapid colonization of the elevated gingival tissues by fibroblasts, and greater connective tissue area occupied by type III collagen during early postoperative healing when compared to wide double flaps [26].

Another explanation for the lack of effect of smoking status can be related to the inclusive definition of smoker patient adopted in this study. A patient who smoked at least 1 cigarette per day was considered a smoker. The inclusion of light smokers may have mitigated, at least in part, the negative effect of smoking on the clinical outcomes.

This hypothesis seems to be corroborated, where patients smoking more than 10 cigarettes per day showed a clear tendency toward a lower CAL gain and PD reduction compared to patients smoking 1 to 10 cigarettes per day. In the light of our results, it was stated that bovine xenograft has yielded significant reduction in probing depth, gain in clinical attachment level (CAL) and significant radiographic bone fill, showing superior effects in treatment of intraosseous defects in smokers and non-smokers.

Graph 1: Comparison of mean difference of Plaque index (PI), Sulcus bleeding index (SBI), Probing depth (PD) and Clinical attachment level (CAL) between SMOKERS and NON-SMOKERS from baseline to 6 months.

Graph 2: Comparison of means of bone gain in smokers from baseline to 6 months.

Graph 3: Comparison of means of bone gain in non-smokers from baseline to 6 months.

Graph 4: Comparison of Means difference of Bone gain between Smokers and Non-Smokers from Baseline to 6 Months.

Variable	Time interval	Smokers	Non-Smokers
		Mean ± SD	**Mean ± SD**
Plaque index	Base line	2.13 ± 0.26	2.15 ± 0.26
	6 months	1.14 ± 0.22	1.09 ± 0.18
Sulcus bleeding	Base line	2.53 ± 0.52	2.53 ± 0.52
Index	6 months	1.47 ± 0.52	1.27 ± 0.46
Probing depth	Base line	8.4 ± 1.6	7.7 ± 1.2
	6 months	3.1 ± 0.5	3.6 ± 0.9
Clinical	Base line	10.0 ± 1.9	10.1 ± 2.5
Attachment level	6 months	5.5 ± 1.9	6.5 ± 2.0

Table 1: Means and Standard Deviations of Plaque Index (PI), Sulcus Bleeding Index (SBI), Probing Depth (PD) and Clinical Attachment Level (CAL) In Smokers and Non-Smokers at Different Time Intervals.

Variable	Time interval	Smokers	Non-Smokers
		Mean ± SD	Mean ± SD
Plaque index	Base line to 6 months	1.06 ± 0.08 p = 0.001(S)	1.06 ± 0.08 p = 0.001(S)
Sulcus bleeding index	Base line to 6 months	1.26 ± 0.06 p = 0.000(S)	1.26 ± 0.06 p = 0.000(S)
Probing depth	Base line to 6 months	5.3 ± 1.3 p = 0.003(S)	4.1 ± 1.1 p = 0.003(S)
Clinical Attachment level	Base line to 6 months	4.5 ± 1.9 p = 0.003(S)	3.5 ± 0.09 p = 0.003(S)

Table 2: Comparison of Means of Plaque Index (PI), Sulcus Bleeding Index (SBI), Probing Depth (PD) and Clinical Attachment Level (CAL) in smokers and non-smokers within the sites from baseline to six months.

Statistical Analysis: Mann-Whitney U test.

S: Significant.

paired "t" test. Statistically significant if $p < 0.05$.

Variable	Smokers	Non-Smokers	p VALUE
	Mean ± SD	Mean ± SD	
Plaque index	1.06 ± 0.08	1.06 ± 0.08	0.467 NS
Sulcus bleeding index	1.26 ± 0.06	1.26 ± 0.06	0.148 NS
Probing depth	5.3 ± 1.3	4.1 ± 1.1	0.028 S
Clinical Attachment level	4.5 ± 1.9	3.5 ± 0.09	0.116 NS

Table 3: Comparison of mean difference of Plaque index (PI), Sulcus bleeding index (SBI), Probing depth (PD) and Clinical attachment level (CAL) between Smokers and Nonsmokers from baseline to 6 months.

Statistical Analysis: Independent 't' test, paired t test.

Statistically significant if $p < 0.05$.

NS: Non-significant.

S: Significant.

	Smokers	Non-smokers		
Time interval	MEAN ± SD	MEAN ± SD	% of recovery	p VALUE
Base line	4.23 ± 0.94	4.53 ± 0.97	53.64	0.000S
Six months	1.30 ± 0.47	2.43 ± 0.57	30.73	0.000S

Table 4: Comparison of Means and standard deviation of Bone Gain in Smokers and Nonsmokers From Baseline to 6months.

Statistical analysis: paired "t" test. Statistically significant if $p < 0.05$.

Time interval	Smokers	Non-smokers	P value
	MEAN ± SD	MEAN ± SD	
Base line to 6 months	2.10 ± 0.40	2.93 ± 0.47	0.002 S

Table 5: Comparison of Mean Difference of Bone Gain in Smokers and Non-Smokers from Baseline to Six Months.

Statistical analysis: independent sample 't' test. Statistically significant if $p < 0.05$.

S: Significant.

EHI score	Smokers	Non-Smokers
Complete flap closure, no fibrin line in the interproximal area	3	5
Complete flap closure, fine fibrin line in the interproximal area	3	4
Complete flap closure, fibrin clot in the interproximal area	5	3
Incomplete flap closure, partial necrosis of the interproximal tissue	1	0
Incomplete flap closure, complete necrosis of the interproximal tissue	0	0

Table 6: Early Healing Index (EHI) Assessment in smokers and non-smokers after 2 Weeks Following the Surgery.

Conclusion

We found that there was significant improvement in clinical parameters i.e., plaque index (PII), sulcus bleeding index (SBI),

probing depth (PD), clinical attachment level (CAL), and bone fill in the sites, before and after periodontal treatment. There was a significant improvement in clinical attachment level gain and bone fill in both the groups treated with bovine xenograft using a single flap approach from baseline to six months. The results of this study point towards the novel direction of current surgical regenerative approaches. Further, long term, multi-center, prospective longitudinal trails are the need of the hour to confirm the finding of this study.

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