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Clinical effects of bovine derived xenograft and platelet rich fibrin in the treatment of periodontal intrabony defects - A comparative study

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Abstract

Background: Intrabony defects with deep pockets occur frequently in periodontitis and represent sites that, if left untreated, are at increased risk for disease progression. Variety of treatment approaches to the restoration of interproximal intrabony defects have been suggested, including the use of different types of bone grafts, guided tissue regeneration, growth factors and a combination of these.

Aim: To evaluate the regenerative potential of xenograft (Bio-Oss®) and autograft (Platelet-rich fibrin) as an adjunct to open flap debridement in patients with

intrabony defects having moderate to severe chronic periodontitis.

Materials and methods: The present study was a split-mouth randomized controlled clinical trial comprising 15 moderate to severe chronic periodontitis patients (mean age: 35.9 years) having at least one pair of bilateral intrabony defect. Group I included 15 defect sites treated with open flap debridement (OFD) + grafting of the defects with platelet-rich fibrin 15 sites whereas 15 defect sites in Group II were treated with open flap debridement (OFD) + grafting of the defects with xenograft (Bio-Oss®). The variables investigated at baseline, 6 and 9 months were the plaque index, the gingival index, modified sulcus bleeding index, probing pocket depth and clinical attachment level. Radiographic assessment of bone fill was evaluated at baseline, 6 and 9 months.

Results: Both treatment groups had significant gains in CAL as well as bone fill, with no significant differences in outcomes between groups. Significant reductions were seen in probing pocket depth, plaque index, gingival index and sulcus bleeding index in both the groups.

Conclusions: The results showed significant improvement in all clinical parameters in both groups. Both PRF and Bio-Oss® were safe to use, without causing any immunologic/antigenic reactions in any of the treated patients. Both the graft materials showed the potential of enhancing the periodontal regeneration.

Keywords: PRF, GTR, OFD, Bio-Oss

Introduction

Periodontitis is defined as "an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with increased probing depth formation, recession, or both."[1]It is characterized by the presence of gingival inflammation, periodontal pocket formation, and loss of connective tissue attachment and alveolar bone around the affected teeth.[2]One of the consequences of periodontitis is appearance of periodontal intrabony defects.[3] Vertical or angular or intrabony defects are those that occur in an oblique direction, leaving a hollowed-out trough in the bone alongside the root.[4]

The ultimate goal of periodontal therapy remains to regenerate the periodontal supporting tissues that have been lost due to disease process.[5] Regeneration is defined as reproduction or reconstitution of a lost or injured part of body in such a way that the architecture and function of lost or injured tissues is completely restored. Periodontal regeneration is a unique orchestrated process that involves biologic events such as cell adhesion, migration, proliferation, and differentiation.[6] Currently, there are variety of surgical treatment modalities available for periodontal regeneration like Guided tissue regeneration (GTR), biomodification of root surface, bone grafts and substitutes, and the use of growth factors.[7]

Amongst these treatment modalities, grafting with biomaterials/bone substitutes have been used with varying success to accomplish the reconstruction of deep intrabony defects[8] since 1923 **(Hegedus)**.[9] Bone grafts may facilitate formation of alveolar bone, periodontal ligament and root cementum through localization of bone-forming cells (osteoneogenesis), providing a scaffold for bone formation (osteoconduction), and by containment of bone inducing substances (osteoinduction).[10]

Bovine porous bone mineral is a natural, porous, cancellous bone mineral derived from bovine bone from which all native organic material has been removed by a chemical low-heat (300°C) extraction process, maintaining physical architecture of bone intact. This anorganic xenograft is commercially available by brand name of Bio-Oss®, Geistlich Pharma AG, Wolhusen,

Switzerland. Chemically, Bio-Oss® is a low crystalline apatite with a 7% content of carbonate.[8] It has an unlimited supply, no additional requirement of donor site, proven safety[11] and showed high degree of biocompatibility.[12]Apart from its good osteoinductive and osteoconductive properties and poor reabsorbability, the bovine bone mineral does not run intolerance or infection risks.[13]

Recently, the attention has shifted towards the use of growth factors to regulate various cell-stromal interactions involved in periodontal regeneration. These growth factors are the vital biologic mediators that can regulate proliferation, chemotaxis and differentiation of locally derived progenitor cells in defect site. Platelet Rich Fibrin was developed in France by **Choukroun et al.** in **2001**.[14] PRF consists of a slowly polymerized fibrin network containing components like cytokines, glycanic chains and structural glycoproteins, and is known to promote both soft and hard tissue healing.[15] It is obtained by gentle centrifugation of peripheral blood and is characterized as being leukocyte and platelet rich and fibrin dense, besides not requiring the addition of any anticlotting agent. PRF is believed to release polypeptide growth factors, such as transforming growth factor-β1, platelet-derived growth factor, vascular endothelial growth factor & matrix glycoproteins (such as thrombospondin1), into surgical wound.[7] Keeping in view the clinical benefits of bovine derived xenograft and PRF, the present study was conducted to evaluate the regenerative potential of xenograft (Bio-Oss®) and autograft (Platelet-rich fibrin) as an adjunct to open flap debridement in patients with intrabony defects having moderate to severe chronic periodontitis.

Materials and Methods

This randomized clinical study was conducted on 15 patients with moderate to severe chronic periodontitis visiting the Department of Periodontology and Oral Implantology, Sri Guru Ram Das Institute of Dental Sciences and Research, Sri Amritsar. The study design was approved by the institutional ethical committee. All the patients were informed about the study and informed consent was signed by each patient.

Fifteen systemically healthy patients (8 women and 7 men, mean age 50.5 years,) with bilateral intrabony defects were enrolled in this study.

The Inclusion criteria were

- Patients with moderate to severe chronic periodontitis.
- Presence of bilateral intrabony defects with a probing pocket depth (PPD) of atleast 6 mm.
- Radiographic evidence of intrabony defects of atleast 3 mm as revealed by periapical films taken with long-cone paralleling technique.

The exclusion criteria were

- Patients having any systemic disease influencing periodontal health.
- Patients who had undergone periodontal treatment in the past six months.
- Alcoholics, smokers, tobacco chewers, drug addicts.
- Pregnant women and lactating mothers.

The participants who fulfilled inclusion and exclusion criteria were included in the study and were divided into two groups viz Group I and Group II each.

Group I: 15 defect sites treated with open flap debridement (OFD) + grafting of the defects with platelet-rich fibrin.

Group II: 15 defect sites treated with open flap debridement (OFD) + grafting of the defects with xenograft (Bio-Oss®).

Presurgical Protocol

The initial preparation phase for treatment consisted of oral hygiene instructions, scaling and root planing.

Occlusal therapy and re-evaluation was done 4 weeks after the completion of this first phase of therapy.

The variables investigated at baseline, 6 and 9 months were the plaque index (Silness and Loe[16]) the gingival index (Loe and Silness[16]), modified sulcus bleeding index (Mombelli[17]), probing pocket depth and clinical attachment level. Radiographic assessment of bone fill was evaluated at baseline, 6 and 9 months using a calibrated grid. The probing measurements were done with a customized acrylic stent that was used as a fixed reference point to minimize distortion.

Preparation of platelet-rich fibrin (PRF)

Platelet-rich fibrin was prepared according to the protocol developed by **Choukroun et al.** in the year **2001**. Prior to the surgery 10 ml of intravenous blood was collected from the patient's antecubital vein and transferred into a sterile glass test tube without any anticoagulant. The test tube was then placed in the centrifuge machine immediately and centrifuged for 10 minutes at 3000 rpm.

The resultant product consisted of three layers:

i) Top most layer consisting acellular plasma.

ii) PRF clot in the middle.

iii) Red blood cells (RBC) at the bottom.

Top most layer was discarded. PRF clot was retrieved from the test tube using sterile tweezers and then separated from the underlying RBC base using a sterile scissors preserving some amount of RBC. After the procurement of PRF, it was immediately transferred to the prepared surgical site.

Surgical Protocol

Pre-surgical patient preparation was done. Patient was made to rinse with 0.12% chlorhexidine digluconate preoperatively. After achieving adequate anaesthesia (2% lignocaine with adrenaline 1:2,00,000), crevicular incisions were given on the facial and lingual/palatal sides reaching the tip of the interdental papilla and an interdental incision. A full thickness mucoperiosteal flap was reflected using the periosteal elevator. After reflection of the flap and exposure of osseous defect, a thorough surgical degranulation of the infected tissue from the osseous defects was done. After thorough debridement, the surgical area was irrigated with normal saline solution.

In group I: Prepared PRF was gently packed into the intrabony defect

In group II: The defect was filled with Bio-Oss® bone graft.

Surgical flaps were repositioned to the presurgical level and sutured with 3-0 non resorbable braided silk reverse cutting suture achieving primary closure. Surgical site was covered with non-eugenol periodontal dressing (Coe-pak®). Post-operative instructions and antibiotics (Amoxicillin 500 mg) and anti-inflammatory drugs (Ibuprofen10 mg) 3 times a day for the next 5 days were prescribed.

After 7 to 10 days, the dressing and sutures were removed. Recall appointments were scheduled at 6 and 9 months after the surgery for soft-tissue evaluation, radiographic evaluation and recording clinical variables.

Statistical analysis

Observational data were analyzed with the Student's t-test. The paired Student's t-test was used to evaluate and establish differences between baseline and postoperative measurements within a group. The unpaired Student's t-test was used to evaluate and establish differences between the group I and groups II at baseline, 6 and 9 months after surgery ($p<0.05$).

Results

All 15 patients enrolled in the study reported for scheduled maintenance and postoperative evaluation visits. A total of 30 defects were treated in 15 patients, 15 defects were treated with PRF and the other 15 defects were treated with Bio-Oss®. All surgical sites showed

uneventful healing. No post-operative complications like swelling, hemorrhage or excessive pain was reported by any patient.

A statistically significant reduction in PI, GI and mSBI was observed in both group I (PRF) and group II(Bio-oss®) (P<0.001) (Table1)

Probing pocket depth (PPD) was significantly decreased as compared to baseline values in both the groups [Table 1], but the difference was not significant between the groups. The reduction in

the PPD for group I(PRF) was 2.69 ± 0.51 mm at 6 months and 3.59 ± 0.38 mm at 9 months while in group II (Bio-oss®) PPD reduction was 2.97 ± 0.47 mm at 6 months and 3.92 ± 0.41 mm at 9 months. With regards to clinical attachment level gain, both groups demonstrated significant improvement over baseline findings [Table 1], but a comparison of treatment revealed no significant difference between the two materials. The gain in CAL for group I(PRF) was 2.57 ± 0.53 mm at 6 months and 3.43 ± 0.43 mm at 9 months while group II (Bio-oss®) showed a gain of 2.75 ± 0.46 mm at 6 months and 3.75 ± 0.39 mm at 9 months.

Radiographic examination revealed a mean intrabony defect fill of 2.36 ± 0.29 mm and 2.45 ± 0.27 mm in group I and group II at 6 months respectively indicating a statistically highly significant difference (p<0.001) from baseline. At the end of 9 months, the radiographic examination revealed a mean intrabony defect fill of 2.94 ± 0.36 mm and 2.98 ± 0.27 mm in group I and group II respectively indicating a statistically highly significant difference (p<0.001) from baseline. This average bone fill corresponds to 46.9 ± 8.32% bone fill at 6 months and 58.2 ± 9.31% at 9 months for group I (PRF) and 49.9 ± 6.81% at 6 months and 60.4 ± 5.74% at 9 months for group II (Bio-oss®) . The comparison between the two groups at 6, 9 months postoperatively revealed the non-significant differences.

Discussion

Complete elimination of infectious processes and regeneration of destroyed connective tissue and bone forms the fundamental basis of periodontal surgical procedures.[18] Current study evaluated the efficacy of PRF and Bio-Oss® to attain periodontal regeneration in intrabony defects in chronic periodontitis patients.

Plaque control remains an important factor in deciding eligibility for surgical treatment and assessing the outcome of periodontal procedure. **Rosling B et al. (1976)[19]** observed that surgical treatment of infrabony pockets resulted in significant bone fill in plaque-free dentitions, while progressive deterioration was noticed in plaque-infected individuals. Thus individuals having unacceptable oral hygiene (plaque index 31 [PI] >1.5) after re-evaluation of Phase I therapy were excluded from the study protocol. Systemically healthy individuals and non-smokers were selected to avoid adverse outcomes of smoking and systemic diseases on periodontal regeneration. [18] The split mouth design was adopted to avoid the inter-patient variability as paired defects in the same subject were treated.[20]

The remarkable reduction in probing pocket depth, gain in clinical attachment and intrabony defect fill seen in PRF group revealed the intensified combined efficacy of growth promoting factors delivered by PRF. PRF, being rich in platelets and growth factors (like PDGF, TGF-β, VEGF), facilitates regeneration of the lost periodontal tissues.[15] Apart from platelets, leucocytes and fibrin in platelet concentrates also have significant roles. Leukocytes have an anti-infectious action and aid in immune regulation. They produce large amounts of VEGF to promote angiogenesis and serve as a biological healing

matrix by supporting cell migration and cytokine release.[20]

The biochemical analysis of the PRF composition indicates that this biomaterial consists of an intimate assembly of cytokines, glycanic chains, structural glycoproteins enmeshed within a slowly polymerized fibrin network. These biochemical components have well known synergetic effects on healing processes.[21] Owing to its dense fibrin matrix, PRF takes longer to be resorbed by the host, which results in the slower and sustained release of platelet- and leukocyte derived growth factors into the wound area. This natural fibrin framework protect growth factors from proteolysis.[22] Thus, growth factors can keep their activity for a relatively longer period and stimulate tissue regeneration effectively. Greater intrabony defect fill (58.2 ± 9.31%) in our study can be attributed to the GFs that enhance periodontal healing. These findings were in accordance with those of Chang YC et al. (2011)[23], Lakshmi P et al. (2014)[24] Saravanan D et al. (2019)[25].

Mathur et al. (2015)[26] compared clinically and radiographically the efficacy of autologous PRF and autogenous bone graft obtained using bone scrapper in the treatment of intrabony periodontal defects and stated that although both gave similar results, using PRF is a safer, cheaper, less technique sensitive, and minimally invasive procedure.

In the present study, Bio-Oss® the natural porous bone mineral resulted in significant improvement of all clinical parameters. The rationale for using bone graft with surgical access is that the promotion of bone formation would also induce new attachment formation along the adjacent tooth root surface.[27] Bio-Oss® is prepared by protein extraction from bovine bone, which results in a trabecular hydroxyapatite structure similar to human cancellous bone and it has the ability to enhance bone formation due to its osteoconductivity.[7] Due to its hydrophilic properties, it facilitates the adsorption of blood cells and proteins.[28] This leads to reliable bone formation. The interconnected porous system of Bio-Oss® appears to have a size and structure conducive to vessel ingrowth. The pores of Bio-Oss® particles prepare appropriate scaffolding for osteogenic cell absorption while the particle's shape make appropriate space for blood vessel penetration. Angiogenesis procedure and its related factors are effective on osteoblast growth and differentiation as well as consequent bone formation.[29]

Further, slow resorption of Bio-Oss® also favors the bone formation. Bio-Oss® exhibited very favorable handling properties which included: ease of delivery to the site, ease of packing the material into the defect, ability of the material to demonstrate an adhesion once placed in the defect, even with significant hemorrhage of the wound site, providing a stable graft, and ability to maintain space once soft tissue closure was achieved. The results of present study are also comparable to the study of Richardson et al.,[11] where the bone fill was reported to be 55.8% using Bio-Oss®.

Van Houdt CI et al. (2018)[30] reported in an invitro study that Bio-Oss® had showed significantly more bone formation compared to CPC/PLGA {calcium phosphate cement (CPC) with polylactic-co-glycolic acid (PLGA) micro-particles}.

The results of the present study confirm that intrabony defects can be successfully treated with PRF and Bio-Oss®.

Conclusion

The present study was aimed at evaluating and comparing the efficacy of bovine-derived xenograft (Bio-Oss®) and Platelet-rich fibrin (PRF) in the treatment of periodontal intrabony defects. The results showed significant improvement in all clinical parameters in both groups.

Both PRF and Bio-Oss® were safe to use, without causing any immunologic/antigenic reactions in any of the treated patients. Both the graft materials showed the potential of enhancing the periodontal regeneration.

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Legend Tables and Figures

Table 1: Comparison of mean values of variables at baseline, 6 and 9 months between PRF and Bio-oss® groups.

	Time	Group I	Group II	P-Value
Plaque Index	Baseline	0.76 ± 0.17	0.75 ± 0.13	0.772
	6 Months	0.60 ± 0.15	0.53 ± 0.18	0.299
	9 Months	0.40 ± 0.12	0.36 ± 0.12	0.481
Gingival Index	Baseline	1.34 ± 0.23	1.44 ± 0.18	0.202
	6 Months	0.81 ± 0.13	0.80 ± 0.14	0.897
	9 Months	0.60 ± 0.12	0.59 ± 0.12	0.773
Modified Sulcus Bleeding Index	Baseline	1.20 ± 0.22	1.14 ± 0.22	0.474
	6 Months	0.58 ± 0.18	0.56 ± 0.19	0.775
	9 Months	0.39 ± 0.07	0.36 ± 0.06	0.279
Probing Pocket Depth	Baseline	7.32 ± 0.96	7.62 ± 0.73	0.355
	6 Months	4.63 ± 1.06	4.65 ± 0.84	0.692
	9 Months	3.73 ± 0.80	3.70 ± 0.66	0.675
Clinical Attachment Level	Baseline	8.14 ± 1.03	8.40 ± 0.72	0.436
	6 Months	5.57 ± 1.17	5.65 ± 0.87	0.832
	9 Months	4.71 ± 0.88	4.65 ± 0.70	0.615
Intrabony Defect Fill	Baseline	5.09 ± 0.41	4.95 ± 0.45	0.387
	6 Months	2.72 ± 0.61	2.49 ±0.52	0.275
	9 Months	2.14 ± 0.59	1.96 ± 0.41	0.353

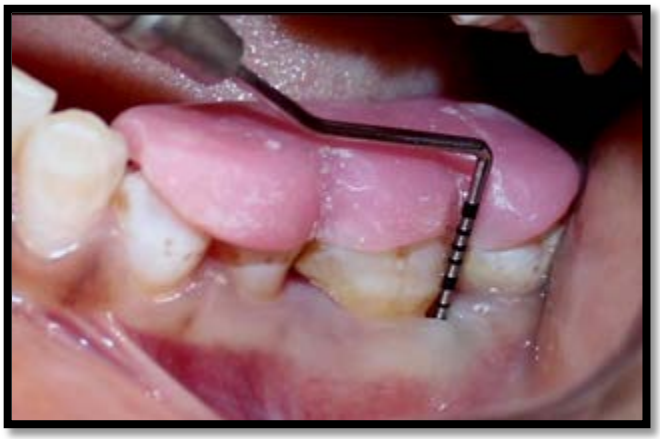

Figure 1: Preoperative Probing Pocket Depth In Group I

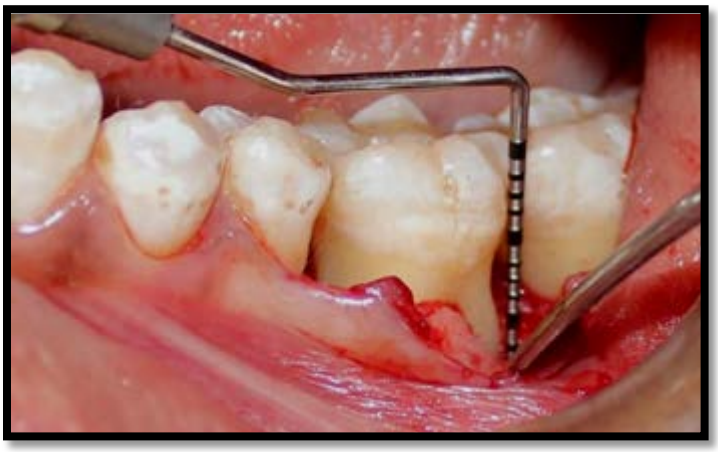

Figure 2: Defect Debridement In Group I

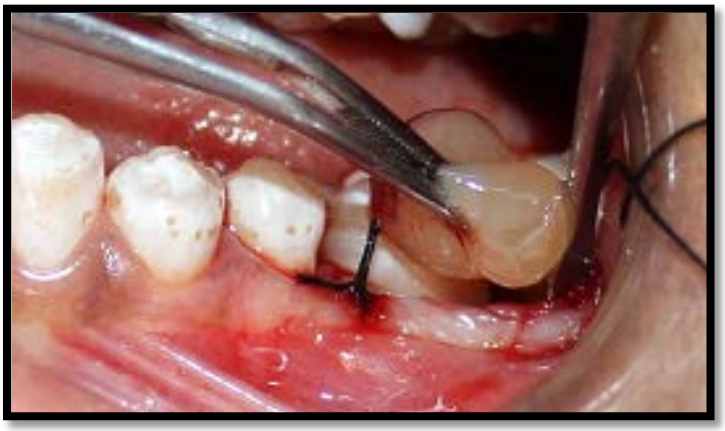

Figure 3: Prf Placement In Group I

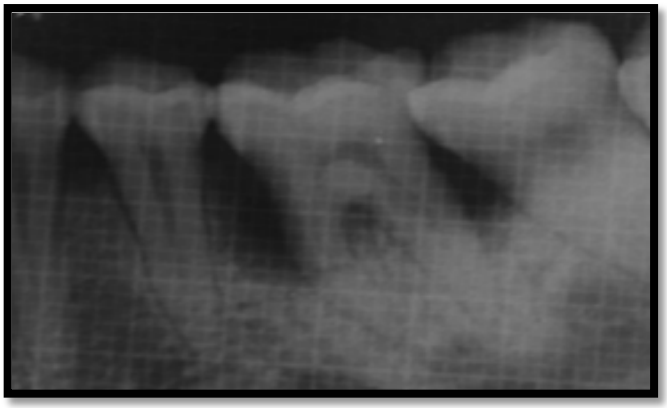

Figure 4: Preoperative IOPA Of Group I

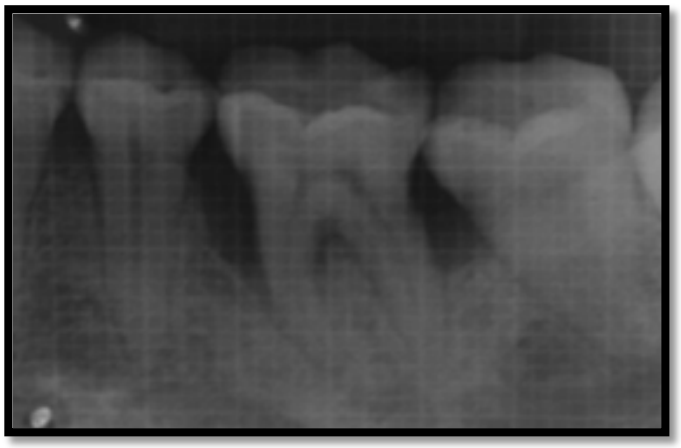

Figure 5: IOPA Showing Bone Fill After 6 Months In Group I

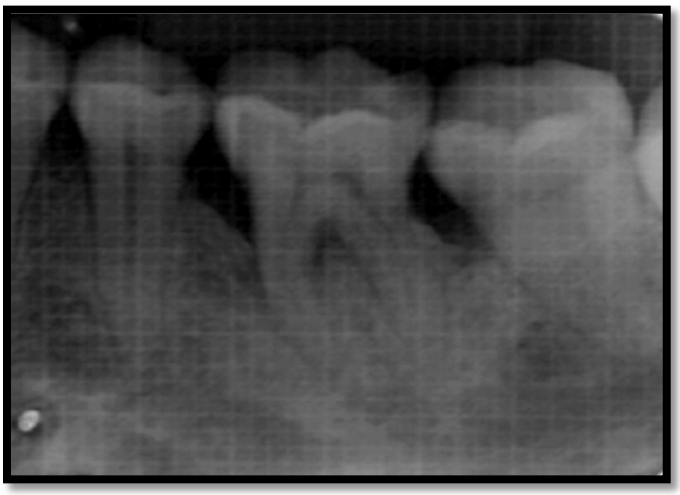

Figure 6: IOPA Showing Bone Fill After 9 Months In Group I

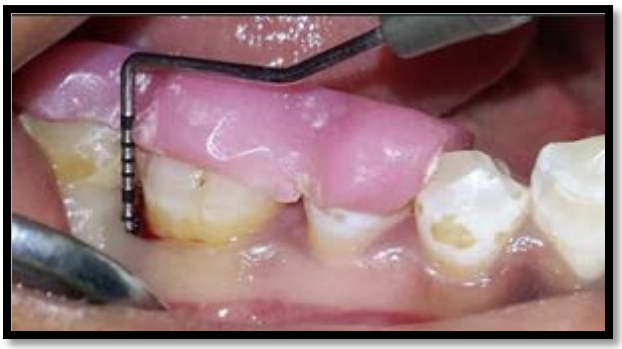

Figure 7: Preoperative Probing Pocket Depth In Group II

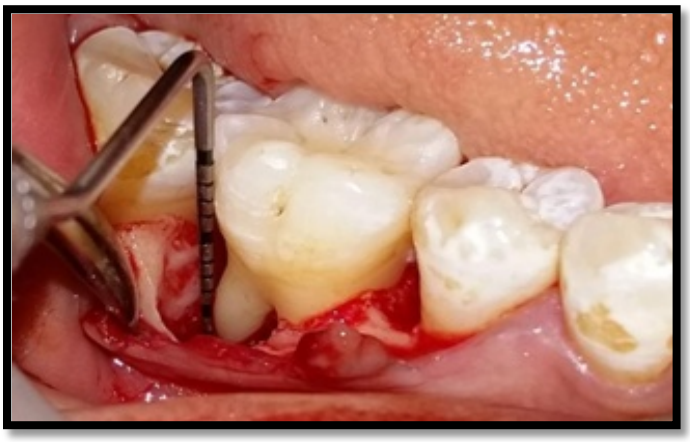

Figure 8: Defect Debridement In Group II

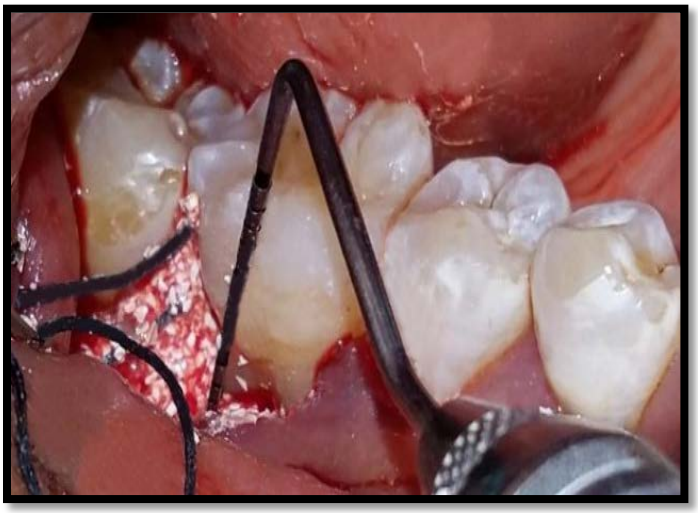

Figure 9: Graft Placement In Group II

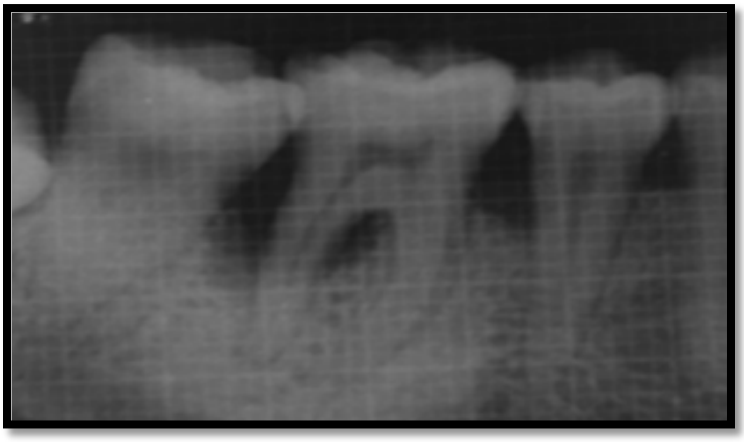

Figure 10: Preoperative IOPA of Group II

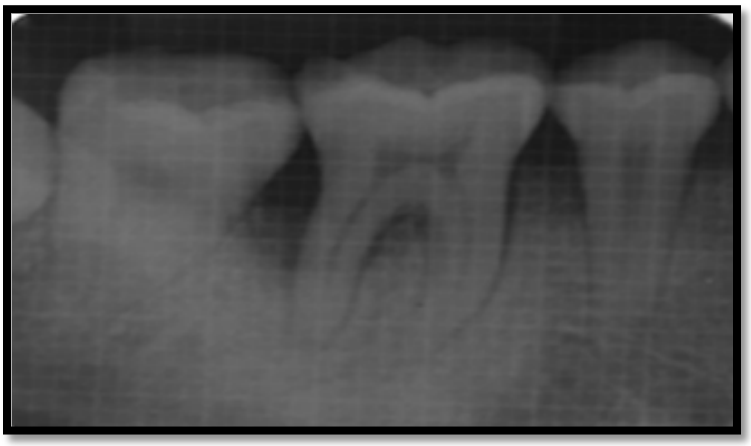

Figure 11: IOPA Showing Bone Fill After 6 Months In Group II

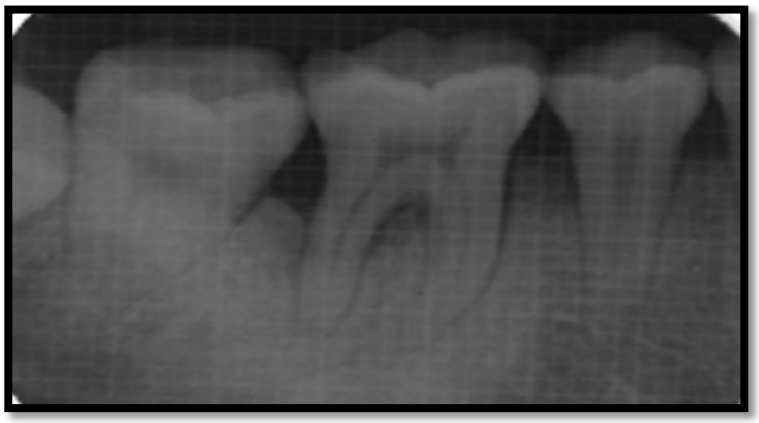

Figure 12: IOPA Showing Bone Fill After 9 Months In Group II

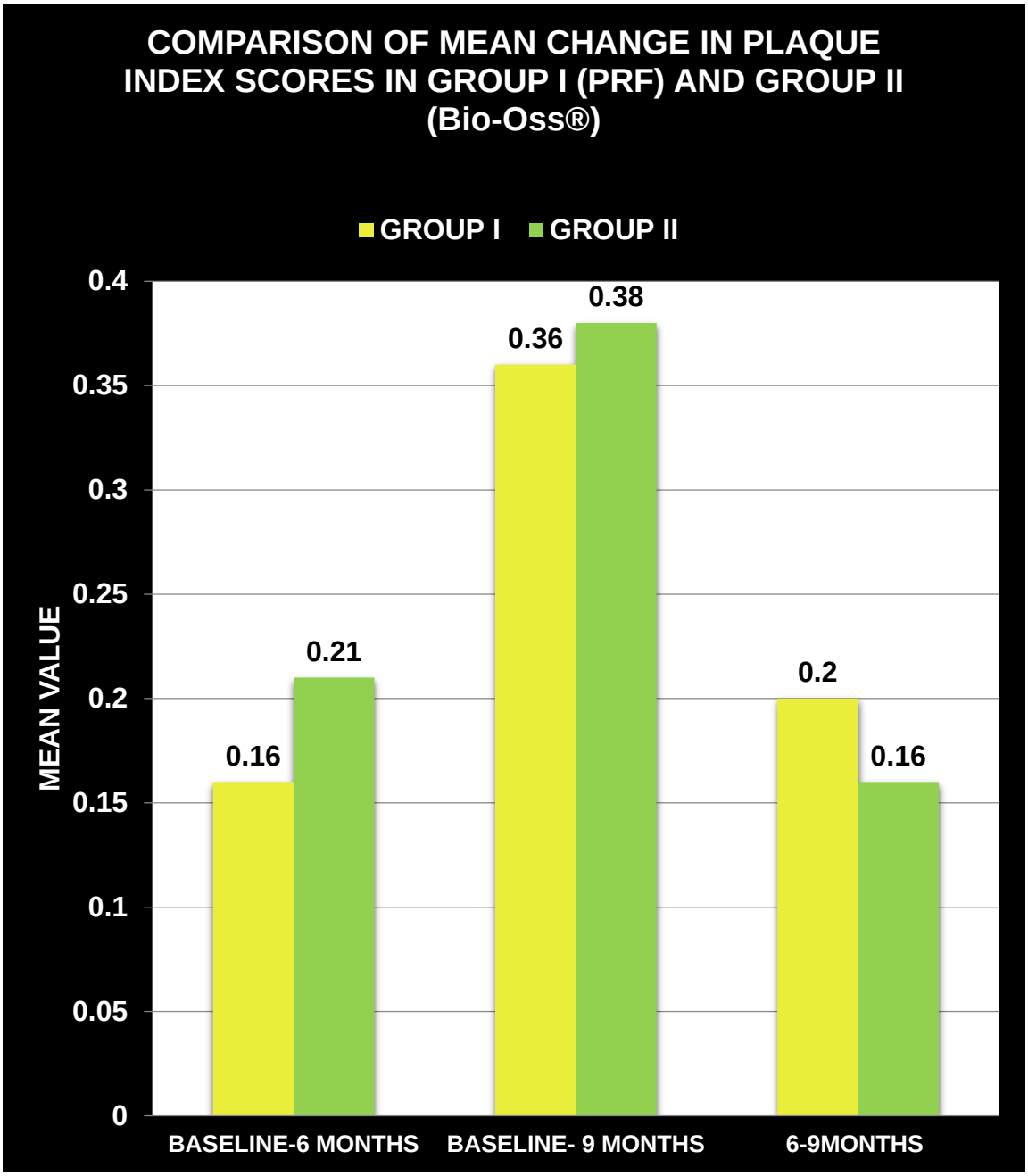

Figure 13: Comparison of Mean Change in Plaque Index In Group I And Group II

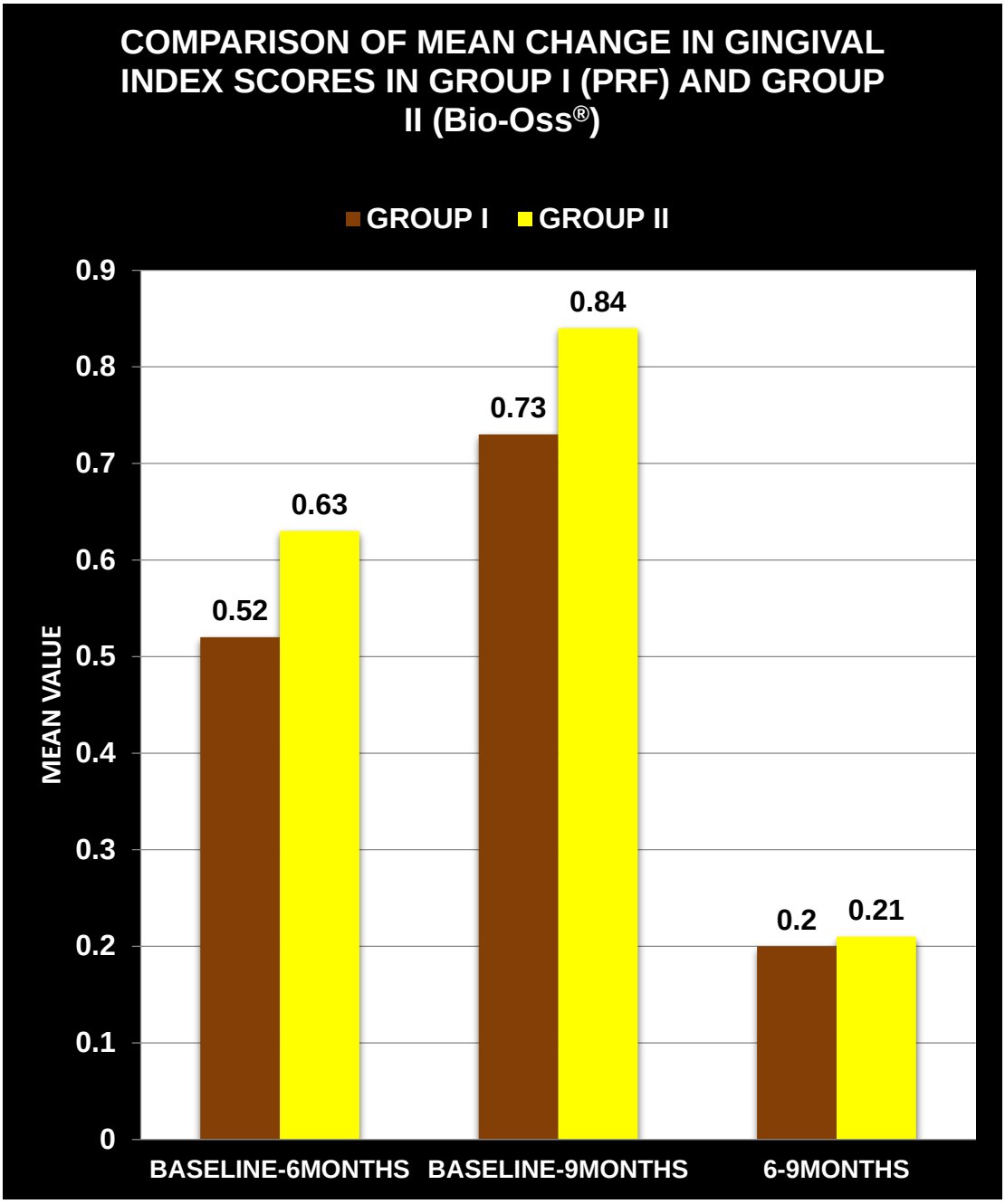

Figure 14: Comparison of Mean Change in Gingival Index In Group I And Group II

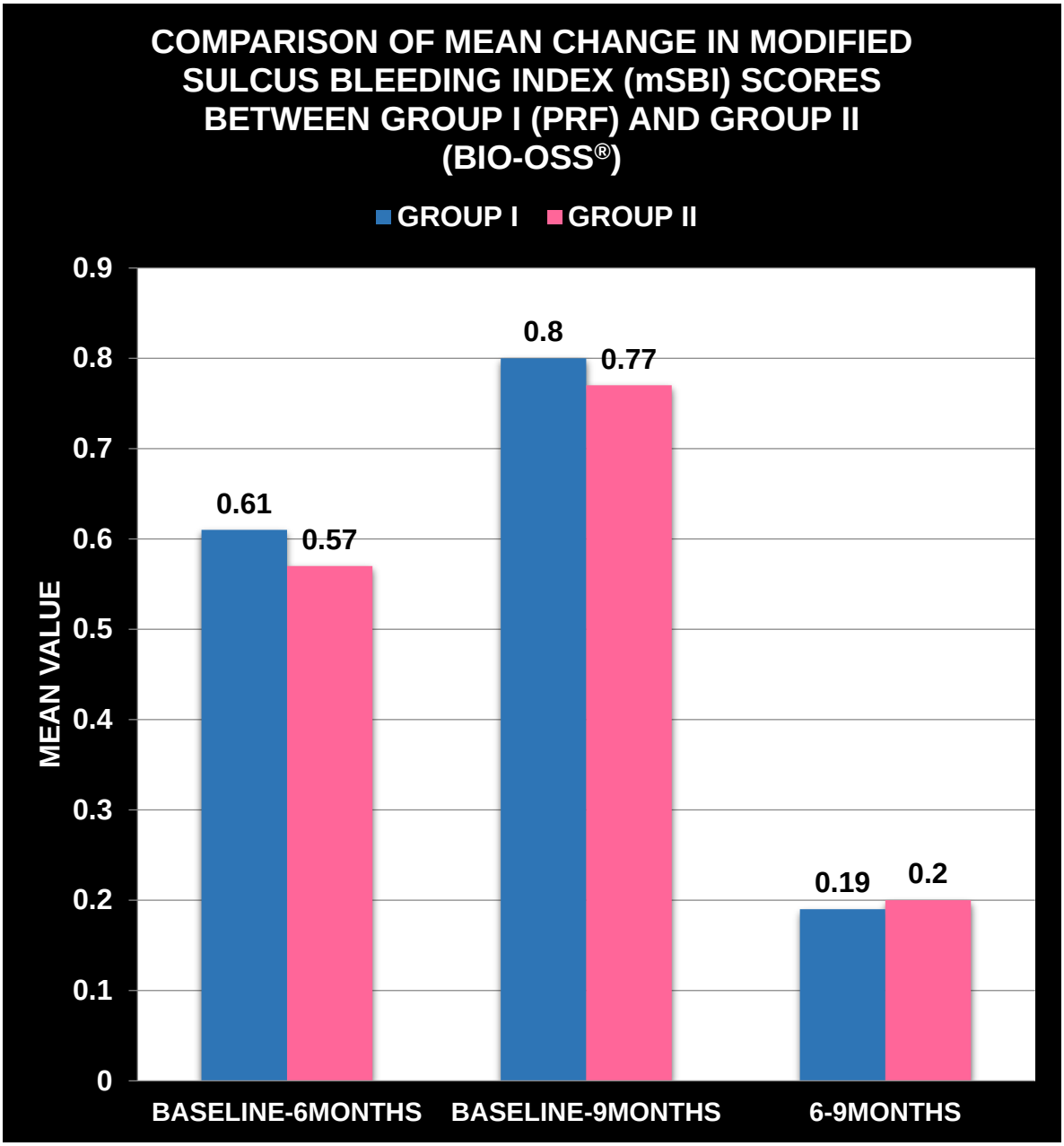

Figure 15: Comparison of Mean Change In Sulcus Bleeding Index In Group I And Group II

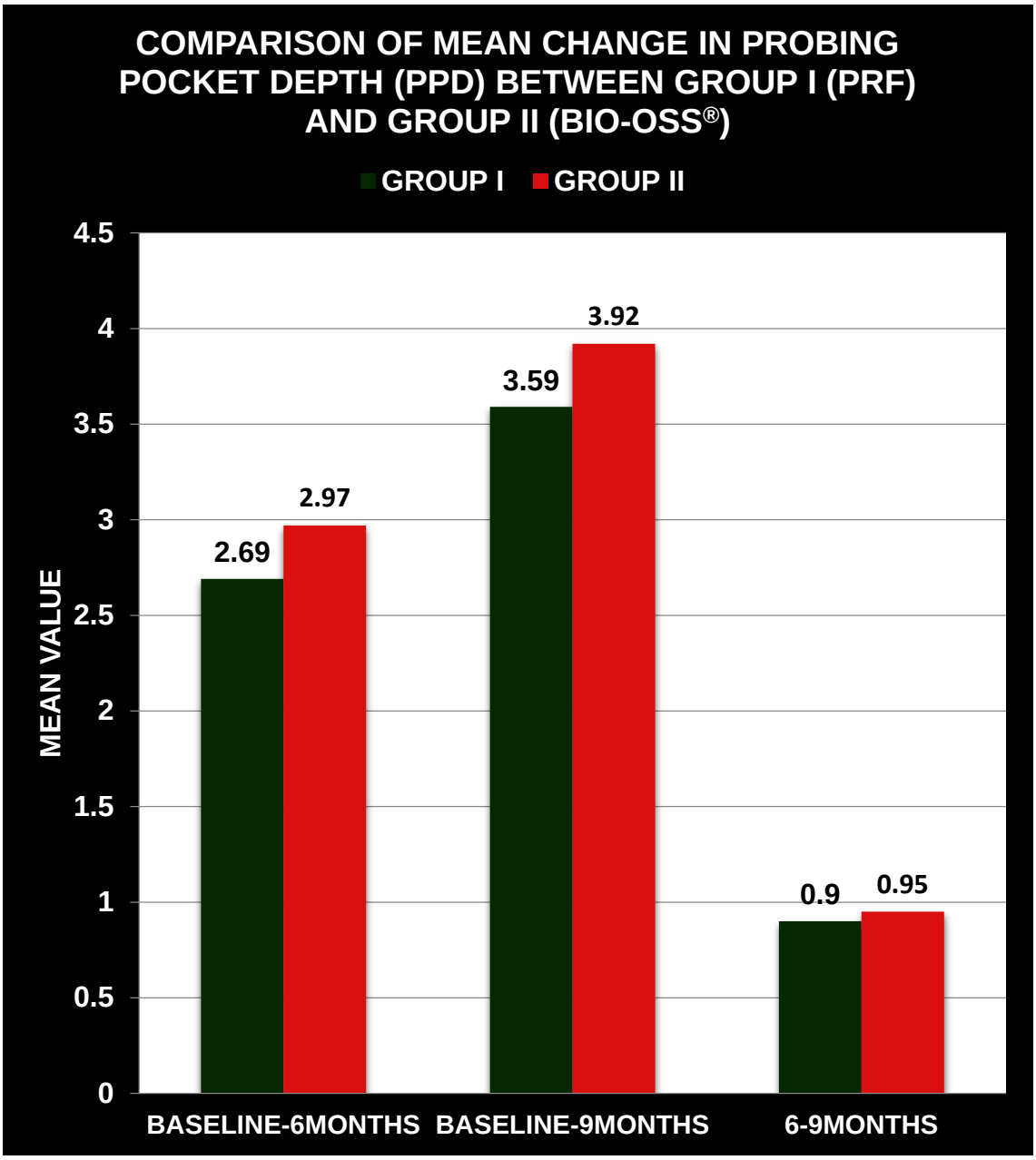

Figure 16: Comparison of Mean Change In Probing Pocket Depth In Group I And Group II

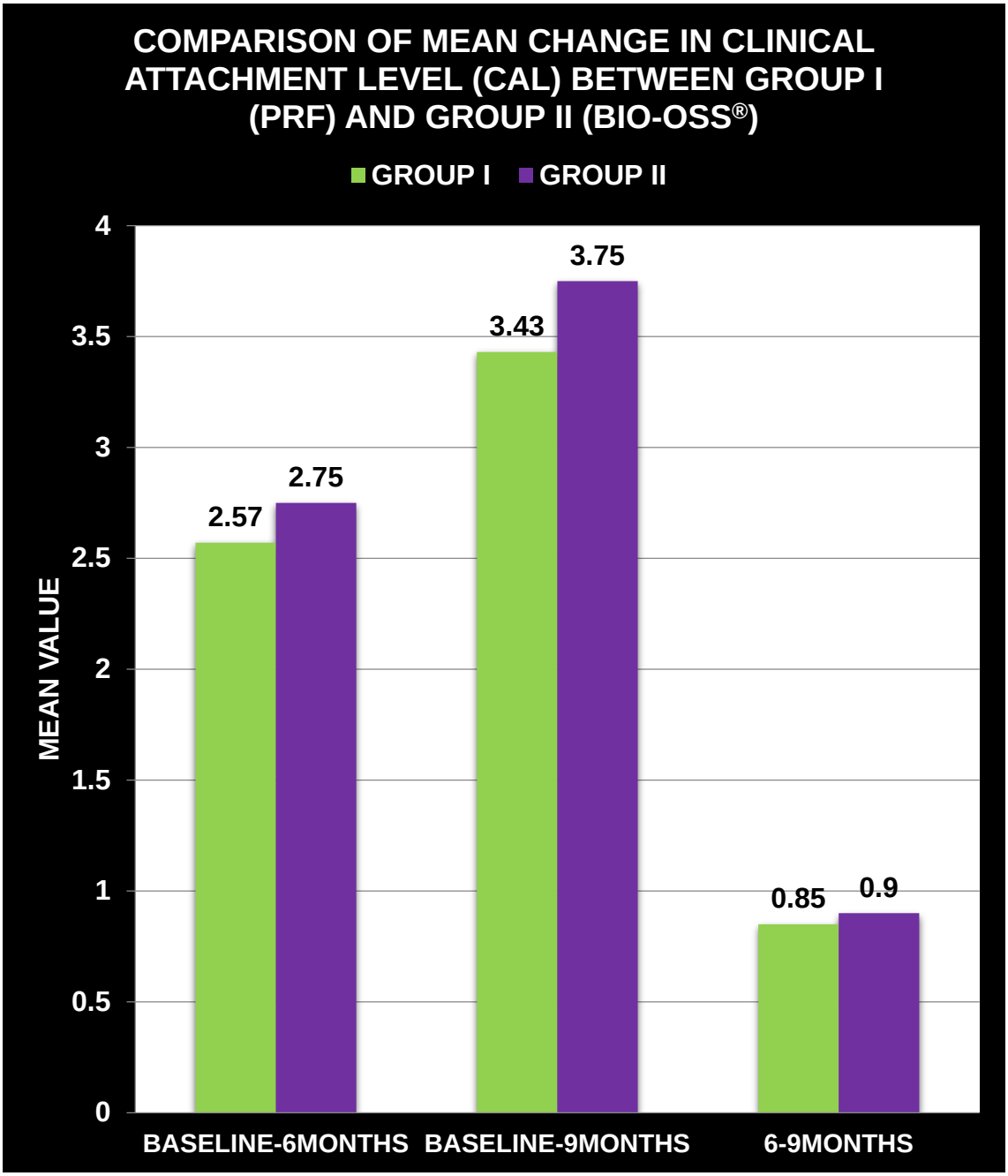

Figure 17: Comparison of Mean Change in Clinical Attachment Level in Group I and GROUP II

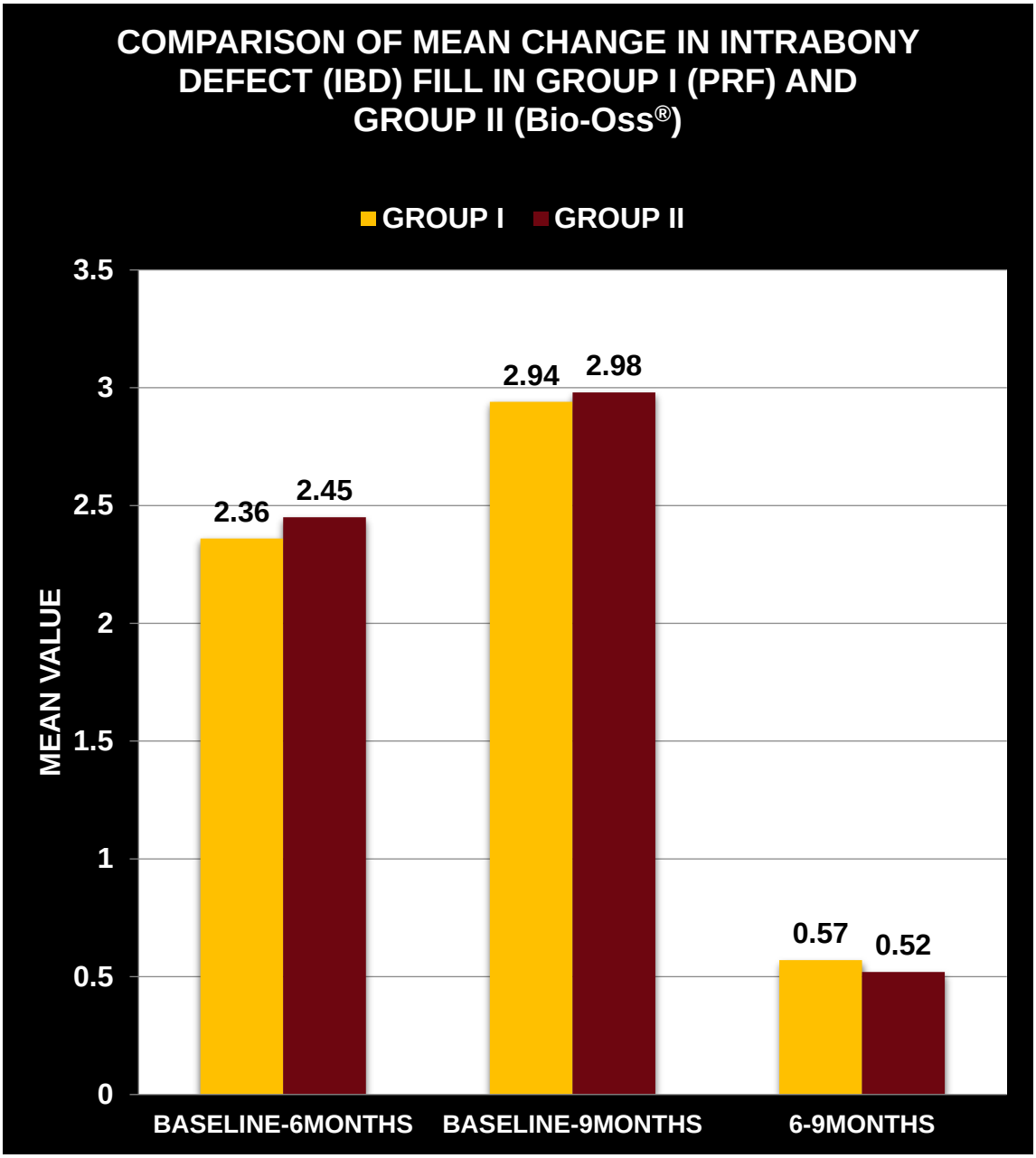

Figure 18: Comparison of Mean Change in Intrabony Defect Fill In Group I And Group II