Original Article

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3 DIMENSIONAL EVALUATION OF CALCIUM CARBONATE (BIOCORAL) AND DEMINERALIZED FREEZED DRIED BONE ALLOGRAFT (DEMBONE) ALONG WITH PLATELET RICH PLASMA IN THE TREATMENT OF PERIODONTAL OSSEOUS DEFECTS

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ABSTRACT:

BACKGROUND: Demineralized freeze dried bone allograft (DFDBA) have been utilized with varying success for the regeneration of periodontium. This study was carried out to compare the effectiveness (qualitatively and quantitatively) of DFDBA with PRP, and coralline calcium carbonate (Biocoral)(Calcarb) with PRP in the treatment of periodontal osseous defects.

METHODS:40 infrabony defects in forty patients were treated randomly with CalCarb with PRP(Group A),DFDBA with PRP(Group B),CalCarb(Group C) and DFDBA(Group D).Clinical parameters such as Plaque Index, Gingival Index, Recession, Probing depth, Clinical Attachment level were recorded at baseline, 3 months, 9 months postoperatively. Preoperative & Postoperative hard tissue measurements (bone defect volume, percent bone fill, bone density) were recorded using Dentascan.

RESULTS: Probing pocket depth reduction were significant at 9 months from baseline for all groups demonstrating 4.10±1.1738 (Group A), 4.20±0.89mm (Group B), 2.75±1.09mm (Group C), 4.20±0.92mm (Group D), respectively. Clinical attachment level gain were significantly improved for all groups A,B,C,D with 3.35±1.2921mm, 3.80±1.09mm, 2.75±1.09mm, 3.70±1.16 mm. Maximum bonegain% was observed in Group A and minimum in Group C. Bone density was maximum in Group B and min in Group C.

CONCLUSION: Group A has significantly better bone gain % as compared to Group B.

KeyWords: Bone Regeneration, Growth Factors, Osseous Defects, Periodontal Regeneration.

INTRODUCTION:

The ultimate goal in periodontal therapy is creation of an environment that is conducive to maintaining the patient's dentition in health, comfort, and function. Periodontal therapy is directed not only at inflammation control but also at pocket reduction and correction of associated bone defects. Moderate to severe periodontal defects are often not amenable to osseous resection without further compromising the support of the involved and adjacent teeth. Regeneration of the lost bone and periodontal attachment improves the support of the tooth and its long-term prognosis.

The shift in therapeutic concepts from resection to regeneration has significantly impacted the practice of

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periodontics in the last quarter of this century. Interest in bone replacement grafts has emerged from the desire to "fill" an intrabony or furcation defect rather than radically resect surrounding intact bone tissue. Bone replacement grafts have been shown to produce greater clinical bone defect fill than flap debridement alone.[1,2,3,4] Histological reports have confirmed their ability to support new attachment in the apical portion of periodontal defects.[5,6,7] The ideal bone replacement graft should be able to trigger osteogenesis, cementogenesis and formation of a functional periodontal ligament. Due to the variable physical and chemical nature among bone replacement grafts, the goal of reproduction or reconstitution of lost periodontal structure (alveolar bone, periodontal ligament and cementum) has been met with varying success. Historically, autogenous and allogeneic bone have been used with some success.[1,2,3,4] Thus focus was on other bone replacement grafts, these materials, synthetically derived or processed from skeletal structures of other species (xenogeneic), are biocompatible and non-organic. Their purpose is to substitute for bone, because often sufficient autogenous bone is unavailable, or harvesting would require additional surgical site and procedure, thus increasing patient morbidity.

Bone replacement grafts are generally osteoinductive, relatively inert filling materials which integrate with new bone. Osteoconductive materials provide a scaffold to allow bone ingrowth and deposition and may result in significant improvement in probing pocket depth and gain in clinical attachment levels. Bone replacement grafts causing osteoinduction (stimulation of phenotypic conversion of progenitor cells) are those that can form osseous tissue, e.g. demineralized freeze dried human bone and bone morphogenetic bone.[8,9] Researchers have demonstrated through numerous animal works that freeze drying of a cortical bone graft induce new bone formation and greatly enhance its osteogenic potential. Natural coral graft substitutes are derived from the exoskeleton of marine madreporic corals. The structure and mechanical property of the commonly used coral, (Porites) is similar to that of cancellous bone. The exoskeleton of these high content calcium carbonate (coral) scaffolds has since been shown to be biocompatible, osteoconductive, and biodegradable at variable rates depending on the exoskeleton porosity, the implantation site and the species. coral grafts also acts as an adequate carrier for growth factors, allow cell attachment, growth, spreading and differentiation.[10] Platelet rich plasma **(**PRP) is a blood derived fraction that contains a high concentration of platelets and growth factors. PRP is obtained by sequestering and concentrating platelets by gradient density centrifugation. It is an autologous concentration of platelets, containing a number of important growth factors such as: platelet-derived growth factor (PDGF), transforming growth factor-b1 (TGF-b1), transforming growth factor-b2 (TGFb2), insulin-like growth factor (IGF), epidermal growth factor (EGF), epithelial-cell growth factor (ECGF) and vascular endothelial cell growth factor {VEGF) It indicate that by adding PRP to bone grafts results both in a faster

radiographic maturation rate and in a higher bone density. It has also been demonstrated that PRP enhances bone regeneration successfully and accelerate soft tissue healing in fresh extraction sites.[11]

MATERIALS AND METHODS:

A 9 months Randomized,Single centered study was carried out in the Department of Periodontics,U.P. Dental College &Research Centre,Lucknow.The study work has been carried out in accordance with the code of ethics of the World Medical Association. The study comprised 40 patients suffering with chronic generalized periodontitis, which included male and female irrespective of their cast, creed and religion who were in the age range of 18-60 yrs. Forty angular bony defects in forty patients were selected and treated following random allocation to one of the treatment modalities. The study period for every subject was 9 months. They were explained about the study procedure in detail and were asked to sign an informed consent as part of the protocol requirements.

1.PRESURGICAL PROCEDURE-A detailed clinical history record,Orthopantomogram(OPG) and Intra oral Periapical(IOPA) X-rays, study casts,complete clinical radiographs,Dentascan,Routine lab investigations- CBC, Blood sugar(random),Australian antigen test were done.

1.1*Phase –I therapy* **-**All the patients underwent phase-I therapy.

1.2*Stent fabrication*-Occlusal stents using self-curing resin were then fabricated in the area of interest and trimmed to the height of contour to serve as a fixed reference guide. A groove corresponding to the infrabony defect was made in the stent to provide a reproducible insertion axis for the probe. The stents were preserved on the study casts throughout the study to prevent distortion.

Clinical parameters were recorded to the nearest mm with the help of a probe guided by the customized stent by a single investigator for each surgical site before surgery (baseline) and post operatively at 3 and 9 months using UNC-15 probe.Oral hygiene status was recorded using the GI and PI.Other parameters recorded were Recession (CEJ-FGM), Probing pocket depth (PPD), Clinical attachment level (CAL).

1.3*Radiological procedure-*Standardized IOPA'S of the defects were taken with a customized film holder.These radiographs were then utilized to assess the defects at baseline, 3, 9 months (Fig-1)

Dentascan assessment-Bone defect morphology was assessedusing 3-Dimensional Computed Tomography(3D-CT)*.The region of interest was focused, and the threshold processing was performed to obtain the images containing bone and teeth. Bone defect morphology was assessed. The measurement was made using the caliper provided with the software, with accuracy to the nearer 0.1mm(Fig-2,3)Volume assessment-Reconstruction of the defect, was done by measuring the defect on the panoramic view and on the buccolingual section of the respective site. With these parameters volume of

the preoperative defect, post-operative bone fill and percentage(%) bone gain can also be calculated.Radiographic assessment-The difference between the pre and post treatment measurements was defined as the radiographic bone fill.

2.COLLECTION OF BLOOD AND PREPARATION OF PLATELET RICH PLASMA(PRP)-20 ml of patients own blood was drawn by veinepuncture of the anticubital vein and was collected in the paediatric blood bags containing citrate phosphate dextrose-adenine(CPD-A) as an anticoagulant in the ratio of 2.8 ml at the Department of Pathology and Blood Bank Department of King George Medical University,Lucknow.For the study the Paediatric blood bag was utilized because the amount of blood withdrawn was less(20 ml), resulting in little amount of obtained PRP, which was although sufficient for the study.Collection of blood in a blood bag maintains its sterility.The collected blood was kept at room temperature for a min of 45 min to minimize the complement activity.

2.1*Preparation of PRP*-PRP was prepared using the centrifuge Refrigerated Floor Model Centrifuge† at Department of Pathology and Blood Bank at K. G. Medical University, Lucknow. Preparation of PRP was done in a closed system using three blood collection bags, which are attached to one another through interconnecting tubes in order to maintain the sterilization (closed system).Preparation of PRP was done by two spins. First spin was for 1700 rpm/10 min and second spin was for 3000 rpm/10 min.In the first spin the R.B.C's were separated and settled down as bottom layer and plasma with platelets as upper layer were transferred to the second bag and first bag with R.B.C.'s was cut off.It was again subjected to second spin in which the poor plasma and plasma rich platelets were separated in the subsequent bags and the bag containing 2-2.5ml PRP was subsequently obtained, which was then stored in an agitator at the temperature of 22^0C.[13,14] The PRP was prepared at K.G.M.U. in the evening a day prior to the surgery and it was transported next day in the morning via closed vehicle to the Deptt of Periodontics,U.P.Dental College and Research Centre.To maintain the viability and the function of the platelets the PRP should be in a continous moving state irrespective of the speed, this was achieved by manually shaking PRP bag.PRP after it is prepared should not be stored for than 5 days because the function of the platelets deteriorates and the viability is not maintained after this period.

2.2*Preparation of PRP Gel*-10,000 unit of powdered bovine thrombin was mixed with 10% calcium chloride.The two preparations are mixed with the PRP kept in a dappen dish to achieve its activation, within 5 to 30 seconds, a gel was formed as the citrate is neutralized and the thrombin activates polymerization of the fibrin and degranulation of the platelets.[13]

3.SURGICAL PROCEDURE: The infrabony defects selected were randomly assigned to either Group A (Biocoral +PRP), Group B (DFDBA+PRP), Group C (BIOCORAL)**‡** , Group D (DFDBA-Dembone)**§** .

Flap preparation:Routine preparation was done.Crevicular incisions were made, and full thickness mucoperiosteal flap were reflected buccally and lingually(if required) to obtain complete access to the defect.Care was taken to preserve as much as interproximal tissue as possible.The lining pocket epithelium was removed so that a fresh connective tissue bed would be in contact during graft placement.Utmost care was taken to preserve the interdental papilla to allow better coverage of the graft material and prevent exposure and exfoliation of the graft and also to aid in better healing.

3.1*Defect preparation*-Meticulous defect debridement and root planning was done.This was followed by removal of granulation tissue from the defect to ensure a clean site for incorporation of the bone graft material.

RESULTS:

The study comprised 40 patients suffering from chronic periodontitis, which included males and females who were in the age range of 18-60 years. Forty angular bony defects in forty patients were selected and treated following random allocation to one of the treatment modalities. The study period for every subject was 9 months. The sites were randomly divided into four Groups to receive **Biocoral with PRP** (Group I) or **DFDBA (Dembone), with PRP** (Group II), **Biocoral** (Group III), **DFDBA** (Group IV)**.**All surgical sites healed uneventfully after the initial surgery. At 3 months and 9 months follow up no clinical signs of inflammation, infection or swelling were evident, indicating that the graft materials appeared to be well tolerated by the periodontal tissues. Particle exfoliation was also not noted with either of the graft material.

The study included the following parameters, which were assessed at baseline, 3 months and 9 months;**Plaque Index,Gingival Index,Bone gain %,Bone density,Recession (CEJ-FGM),Probing pocket depth.Clinical attachment level.**

* The observations obtained for all the above parameters were put to statistical analysis using paired and unpaired Student's't' test.

Plaque Index at 3 months, 9 months - There is significant reduction in all groups (I, II, III, IV) in Plaque Index at 3 months, 9 months.

Comparison of Plaque Index in different Groups at 3 & 9 months*:Plaque Index reduction is maximum in Group IV then in Group III then in Group II and min in Group I at 3 months. I vs. III, I vs. IV, II vs. IV are significant and others are non-significant.Plaque Index reduction is maximum at Group I, then Group IV, then Group II, and min in Group III. All comparisons were non-significant.*

***Gingival Index at 3&9months*-**There is significant reduction in all groups (I, II, III, and IV) in Gingival Index at 3 month and 9 month.

Comparison of Gingival Index in different Groups at 3 & 9 months

1 *Gingival Index reduction is maximum is Group II than in Group*

I then in Group IV and min in Group III at 3 months. All comparisons are non significant.

2 *Gingival Index reduction is maximum at Group II, then Group IV, then Group I, and min in Group III. All comparisons were non-significant. Group II vs Group III is significant.* **bone gain %**

Bone gain % in different groups at 9 months

***Baseline values*:**The bone volume values for BIOCORAL+PRP ranged from 8 to 36mm^3 and the mean score was23.35±8.53mm^3 For DFDBA +PRP sites ranged from6 to 36 mm^3 and the mean score was 23.25±9.75 mm^3.BIOCORAL sites ranged from 18 to 56 mm^3 and the mean score was26.60±11.35 mm^{3}and the sites treated with DFDBA ranged from 18 to 42 mm^{3}with a mean score of 26.35±6.51 mm^3.

Post-operative measurements (at 9 months)-At 9 months, bone fill volume for BIOCORAL sites ranged from 6 to 23.62 mm^{3}with a mean of 17.36±6.39 mm^3, and 9 to 15 mm^3 for the DFDBA+PRP Group with a mean of 12.80±5.12 mm^3, and BIOCORAL ranged from 7.5 to 22.5 mm^3 with a mean score of11.83±4.62, DFDBA ranged from 12 to 27 with a mean score of 16.30±6.07 mm^3.Percentage bone gain at post-operative is maximum in Group I than in Group II and Group IV and minimum at Groups III(Fig-6)***Bone Density in different Groups at 9 months-***Bone density measurement is maximum in Group II than Group I, Group IV and Group III. All the Groups showing significant value. Bone density is significantly less at post-operative site in Group I, II, III and IV. as compared to alveolar bone density adjacent to the infrabony defect. ***Different variables in different groups at 3 & 9 months (PPD)***-At 3 month PPD is maximum in Group II, than in Group IV, than in Group I and minimum Group III, all comparisons are non-signature.AT 9 month PPD is maximum in Group II&IV, than in Group I and minimum at Group III at 9 month.***Different variables in different groups at 3 month & 9 month (CAL)***-CAL change is maximum in Group II, than in Group IV than Group I and minimum in Group III at 3 month. Difference - II vs. III and III vs. IV is significant at 3 month and other are non-significant.At 9 month CAL change is maximum in Group II than in Group IV, than in Group I and minimum in Group III. Group II vs. III is significant at 6 month and other comparisons are non-significant. ***Different variables in different groups at 3 month & 9 month (Recession-CEJ to FGM)*** Recession (CEJ to FGM) is maximum in Group–I, than in Group-III than the Group II and minimum in Group IV.Recession(CEJ to FGM) is maximum in Group–I than in Group-III, than in Group – IV and minimum at Group – II. But the difference is not significant.

DISCUSSION:

The aim of any periodontal therapy is to arrest and control the infection and ultimately to regenerate the lost supporting apparatus of the tooth.[15] Allogenic freeze-dried bone was introduced to periodontics in the early 1970s, while DFDBA bone gained wider application in the late 1980s.[1,2,3,4,44]

DFDBA has a better bone formation ability over FDBA because demineralization results in exposure of bone morphogenetic proteins(BMP's) which stimulate the bone formation through osteoinduction by inducing pleuripotent stem cells to differentiate into osteoblasts.[17-20] Urist et al[46] (1983) established that factors present in DFDBA stimulated migration, attachment and proliferation of cells at the healing sites along with chondroblastic and osteoblastic cell differentiation activity.[17-19]Human histologic evidence indicates that DFDBA can promote the formation of new attachment apparataus on root surfaces.[21] Schwartz et al[22] found that the different DFDBA preparations varied significantly in particle size and inductive properties.Particle size between 250µ -750µ haveshown to promote osteogenesis,[23] Fucini et al[24] however concluded that there was no statistically significant differences in bony fill between defects grafted with different particle sizes of DFDBA when used in humans.The probability of HIV transfer following appropriate DFDBA preparation has been calculated to be 1 in 2.8 billion.[25] At this time, DFDBA remains the only bone replacement graft proven to result in periodontal regeneration in a controlled human histological study.[26] Biocoral is calciumcarbonate obtained from a natural coral,(genus porites), and is composed primarily of aragonite(Crystalline form> 98% CaCo3).Its characteristic features include an open porosity(all the pores communicate between them), volume, the size, the thickness of the porous walls and structural regularity of pores. These characteristics allow a blood cells regulation and a penetration in the core of the graft by bone marrow cells(blood, anions, cations)[27] Elements such as fluoride,strontium andmagnesium are present in slightly greater than 1% total concentration, other minerals present are sodium, potassium,phosphorus(in the form of phosphates), and water.Fluorine in proper quantity increases bone formation by direct effects on proliferation of cellular precursors of osteoblasts.[10] Biocoral is provided as granules 300-450µ,300-450µ in diameter of this material is indicated for periodontal bone defect.[10] The physical attributes of the Biocoral make it appear to be similar to other synthetic bone replacement bone graft material such as β tricalcium phosphate, porous hydroxyapatite, but it is chemically different from these.It has a high osteoconductive potential.No fibrous encapsulation has been reported.[28]It appeared to have good haemostatic properties and is not readily displaced from the treatment sites once a calCarb/blood coagulum has formed.[10] The natural calciumcarbonate constituent in this material may be advantageous in providing a carbonate phase which is essential for the bone formation, whereas other allogenic and alloplastic types of bone replacement graft materials need to undergo a surface transformation to carbonate by heating process from hydroxyapatite during manufacturing process which can result in the alteration of chemical properties of the material thus affecting their osteoconductive potential[29-33,34] .It is not altered by heating into a different, non resorbable chemical structure[29-32,35] Researchers demonstrated that coralline calciumcarbonate gives comparable results to other bone replacement grafts

with significant gain in clinicalattachment,reduction of probing depth and defect fill.[27] They have reported its successful use for the treatment of intrabony defects and ridge augmentation[36,37] PRP is an autologous concentration of human platelets in small volume of plasma.It has polypeptide growth factors(PGFs), who arebiologic mediators which have the ability to regulate cell proliferation, chemotaxis and differentiation. There are at least seven fundamental polypeptide growth factors secreted actively by platelets to initiate wound healing.There are 3-isomers of PDGF (AA,BB,AB), two transforming growthfactor-β (TGF- β) (β1 &β2), IGF-Insulin growth factor, vascular endothelial growth factor(VEGF), andendothelial growth factor(EGF).Among the PGFs known upon activation,TGF-β facilitates wound healing under inflammatory conditions.Polypeptide growth factors, such as PDGF and TGF-β, are known to be abundant in the α- granules of platelets.The cells are activated byconcentrating the platelets.[11] When the platelets in PRP are activated by thrombin, they release growth factors and other substances that serve to accelerate the wound-healing process by increasing cellular proliferation, matrix formation, osteoid production, connective tissue healing, angiogenesis, and collagen synthesis.[34] Researchers in vitro studies[39] showed that PRP stimulated the proliferation of periodontal ligament and osteoblastic cells, whereas epithelial cell proliferation was inhibited.It was seen that because of its fibrinogen content,PRP reacts with thrombin and induces fibrin clot formation, which in turn, is capable of upregulating collagen synthesis in extracellular matrix and provides a favorable scaffold for cellular migration and adhesion.[40]The fibrin component of PRP gel works as ahaemostatic agent aiding in stabilizing the graft material and the blood clot[41-43] and it adheres to the root surface and may impede the apical migration of epithelial cells and connective tissue cells from the flap.[43]PRP acts as an anti-inflammatory agent by producing regulated on activation normal T-cell expressed and secreted(RANTES) blocking monocyte chemotactic factor-I release from monocytes..It has been demonstrated that the application of PRP to the site increasesthe concentration of platelets(PDGF and TGF-β) by upto 338%.With dentascan we were able to calculate the bone gain percentage(%) and the bone density.Significant change in GI in all groups at 3& 9 months was present.Recession increase was significant in all groups at 3& 9 months except in Group D at 3months, which could be due to the result of the reflection of the flap during during the surgical procedures, supported by grafts rather than the bone that can result in reduction of vascular supply especially at the flap margins resulting in recession.[44] However, Renvert et al[45] observed recession/soft tisuue craters in 64% cases, after treatment of infrabony defects 3 weeks postoperatively.[45] There was a mean reduction in the values of PPD in all the four groups suggesting that the material were biocompatible.Inter group comparison was done for Plaque reduction,Gingival index,Recession,PPD,CALWhen Group B was compared with Group C, Group B had PPD and CAL reduction because factorspresent in DFDBA stimulated

migration, attachment and proliferation of cells at the healing sites along with chondroblastic and osteoblastic cell differentiation activity.[46] Studies have shown that DFDBA hasosteogenicpotential.[17-19]

Preoperative bone volume of the defect was calculated by measuring the length, width and depth of the defect and reconstructing these from images/sections on the dentascan.After 9 months measurements of the same site was done.(Fig-4,5,6)Higher bone gain % in Group A was also due to the additive effect of PRP with the graft due to the release of growth factors compared to bone gain % in Group C in which PRP was not added. Though Biocoral is an osteoconductive material but it becomes osteoinductive when it is applied in combination with PRP.

CONCLUSION:

Calcium Carbonate has significantly better bone gain % as compared to DFDBA with PRP.

FOOT NOTES

3-Dimensional Computed Tomography (3D-CT)-(Siemens, Emotions 6)*

Centrifuge Refrigerated Floor Model Centrifuge (Cryofuge-Hereaus-Germany)†

Biocoral(Inoteb,SaintGonnery,France)‡

Dembone(Pacific Coast Tissue Bank)§

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FIGURES:

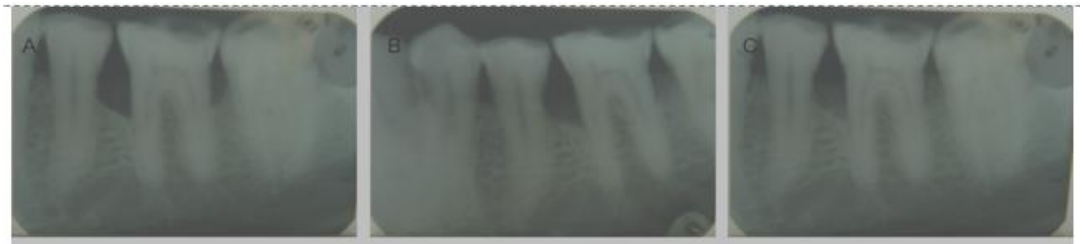

Figure 1. Placement of Dem Bone along with PRP at sites 45 and 46,46 and 47
A. Preoperative view of baseline B. Post operative view after 3 months C. Post operative view after 9 months

Placement of Biocoral graft along with PRP between 35 and 36,36 and 37.
D. Preoperative view at baseline E. 3 Months post surgery F. 9 Months post surgery

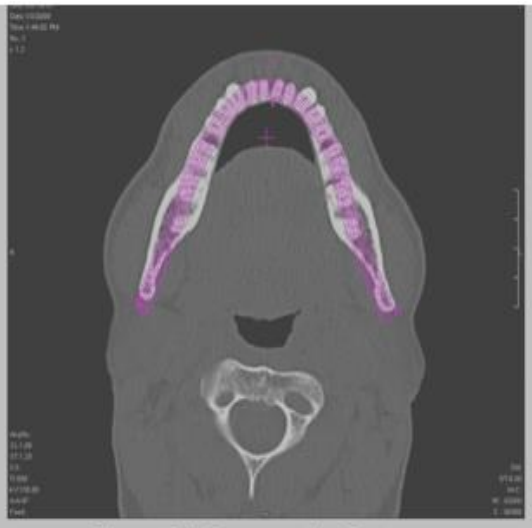

Figure 2A. Preoperative Dentascan
Multiple left to right sections of the mandible for panoromic view

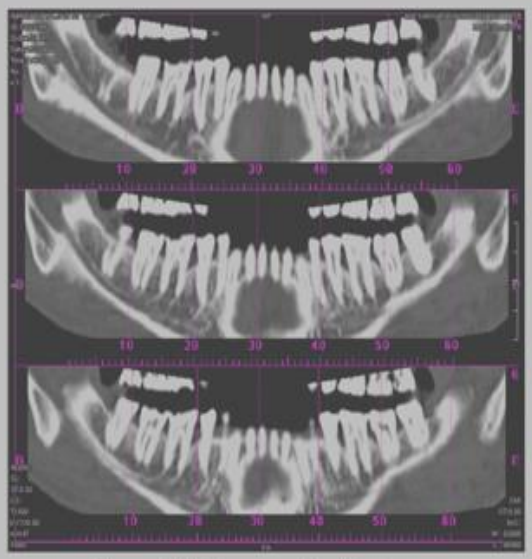

Figure 2B. Preoperative Dentascan
Section of panoromic view

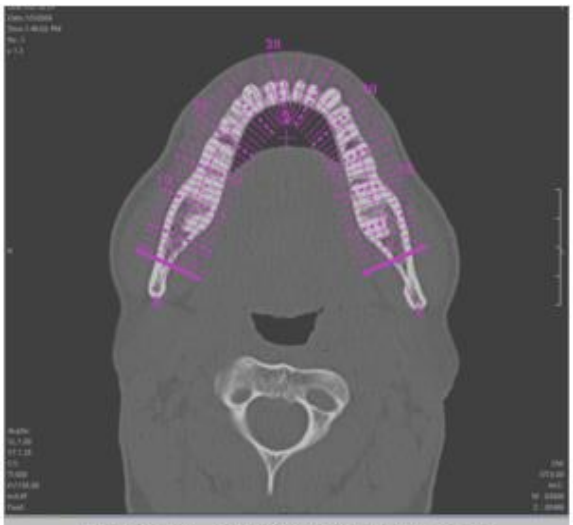

Figure 3A. Pre operative Dentascan
Buccolingual sections of the site involved

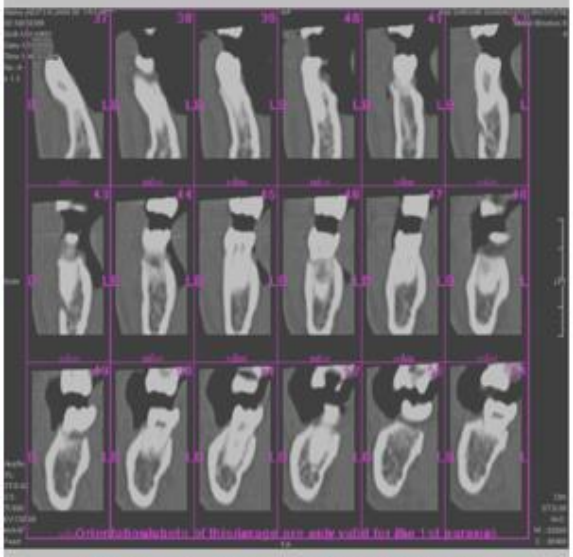

Figure 3B. Pre operative Dentascan
Buccolingual sections of the site involved

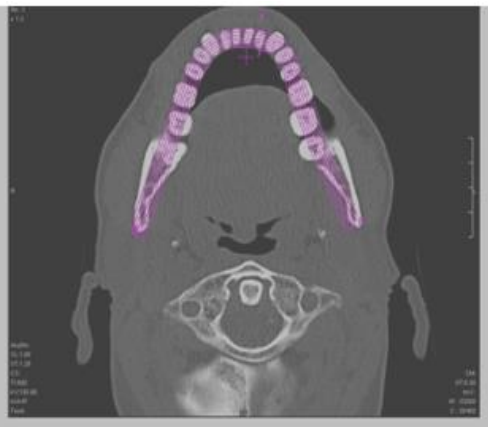

Figure 4A. Post operative dentascan after 9 months
Multiple left to right sections of the mandible for panoromic view

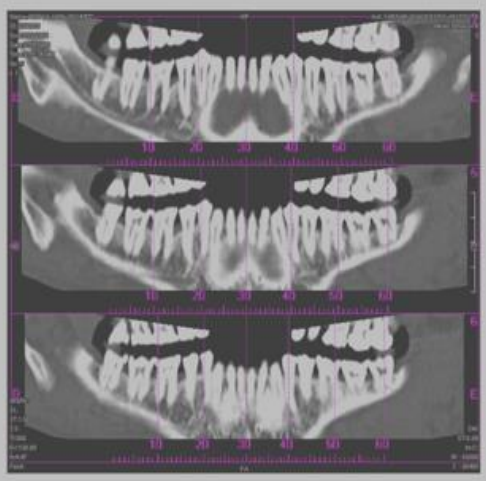

Figure 4B. Post operative dentascan after 9 months
Section of panoromic view

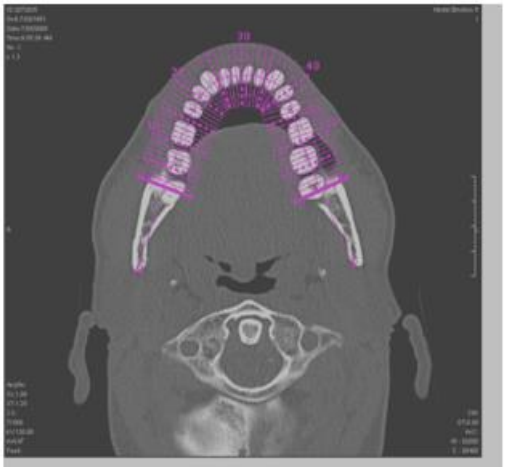

Figure 5A. Post operative dentascan after 9 months
Buccolingual section

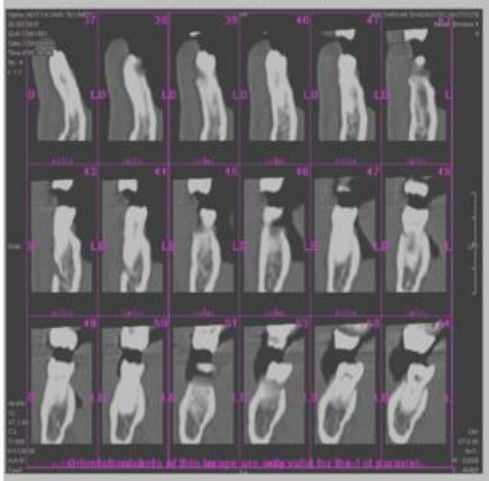

Figure 5B. Post operative dentascan after 9 months
Buccolingual sections of the site involved

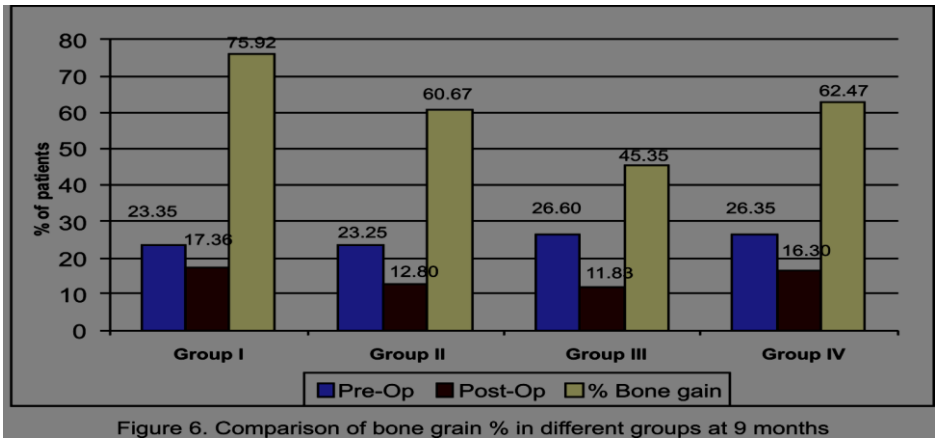

Figure 6. Comparison of bone grain % in different groups at 9 months