

Comparative Evaluation of Clinical and Radiological Parameters Following the Use of Biphasic Alloplastic Material With Amniotic Membrane/ Collagen Membrane for the Management of Periodontal Intra-bony Defects – a Prospective Double Blinded Randomised Controlled Clinical Trial

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Abstract

Background: The concept of periodontal regeneration has been revolutionised since the introduction of growth factors and bioactive bone substitutes which ensures optimal regeneration of the diseased periodontium. The aim of the present study was to evaluate the efficacy of Amniotic membrane + Biphasic Calcium phosphate as compared to Collagen membrane + Biphasic Calcium phosphate for the management of periodontal intrabony defects

Methods: 50 systemically healthy patients with localised moderate to severe periodontitis, sites which had a Probing Pocket Depth (PPD) ≥ 6 mm and an intrabony component of ≥ 3 mm as detected on Intra oral periapical radiographs (IOPAR) and bone sounding were recruited based on specific inclusion and exclusion criteria. They were randomly allocated by computer generated tables to CM+BiCP and AM+BiCP groups. The amount of bone fill and changes in Probing Pocket Depth, Clinical Attachment Level were measured at baseline and six months.

Results: The results of the present study showed a mean reduction in the PPD of 2.89 ± 0.69 mm in the CM+BiCP group and 2.95 ± 0.57 mm in the AM+BiCP group and CAL gain of 2.60 ± 1.43 mm in CM+BiCP group 3.18 ± 1.13 mm in the AM+BiCP group at 6 months follow-up with no statistical significance between the groups. In terms of Defect resolution, $98.62 \pm 6.51\%$ was achieved in CM +BiCP group and $98.25 \pm 7.21\%$ in AM +BiCP group.

Conclusion: Within the limitations of the present study, it can be concluded that AM can be used as a barrier membrane, in conjunction with Biphasic calcium phosphate, and provides comparable results to Collagen membrane with Biphasic calcium phosphate when used in the management of periodontal intrabony defects.

Trail registration: The study protocol was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (IEC/18/DEC/145/51) and was registered in the Clinical Trial Registry of India [Ref No: CTRI/2020/03/0240075].

Background

There has been a significant progress in various aspects of periodontal therapy in the last two decades, from resective periodontal surgical procedures to techniques aimed at regeneration and reconstruction of the lost periodontium. The concept of periodontal regeneration has changed from being passive to an active protocol with the introduction of tissue engineering and biomimetic concepts. It utilises mechanical, cellular and biologic mediators to facilitate reconstruction/regeneration of a particular tissue¹.

Anton Sculean, in 2017 put forth clinical protocols, that have shown to enhance periodontal regeneration and clinical outcomes in periodontal intrabony and class II furcation defects, which includes: (a) Use of

Enamel Matrix Proteins (b) Guided Tissue Regeneration (c) Use of bone grafts enriched with growth factors (Or) Combination therapy².

Guided tissue regeneration with barrier membranes has been demonstrated to be effective in preventing epithelial and gingival connective tissue cells from migrating onto the instrumented root surface³. The primary outcomes in the treatment of intrabony defects achieved by guided tissue regeneration are (i) increase in functional tooth support (clinical attachment and bone levels); (ii) reduction in pocket depth; and (iii) minimal gingival recession⁴.

Various nonresorbable barrier membranes have been employed in animal and clinical studies however, membrane exposure and subsequent contamination and the need for second surgical procedure to remove the membrane are the most prevalent problems associated with the use of nonresorbable membranes. Among the various natural and synthetic biodegradable membranes, collagen based membranes are frequently applied⁵.

Type I collagen (from varied sources) serves as a potential barrier for use in GTR procedures. It acts as a, a) major extracellular macromolecule of the periodontal connective tissue, b) aids in fibroblast chemotaxis, c) cell occlusion d) weak immunogen⁶. Combining CM with bone substitutes may prevent soft tissue collapse, especially in non-contained infrabony defects, and thus ensure space maintenance (Bunyaratavej and Wang, 2001)⁷. However, collagen membrane act mainly as barriers, and they are considered biologically inactive. Fetal membranes as biodegradable materials have been employed in medicine for decades. Amniotic membrane (AM), the innermost layer of fetal membranes, was first used for transplantation of skin in 1910. AM serves as a fibrillar scaffold and acts as a reservoir of stem cells, modulates angiogenesis and promote wound healing⁸.

In an attempt to further improve the clinical outcomes of GTR techniques, particularly in unfavourable and large defects, it has been suggested by clinicians the use of bone grafts with the barrier membrane to obtain an additive effect; this association is called Combined Periodontal Regenerative Therapy (CPRT) ⁹. This approach may lead to the assemblage of different regenerative principles, such as osteoconductivity and osteoinductivity provided by the bone grafts and space provision, wound stability provided by the barrier membranes.

Hence a combination of AM/CM with bone substitute may positively influence the treatment of periodontal intrabony defects by enhancing wound healing and promoting robust periodontal regeneration. The aim of the present study was to evaluate the efficacy of Amniotic membrane + Biphasic Calcium phosphate as compared to Collagen membrane + Biphasic Calcium phosphate in the management of periodontal intrabony defects.

Materials And Methods

Trial design:

The study was designed as a prospective double-blinded randomized controlled clinical trial with two arm parallel group design. Patients visiting the out-patient department of Periodontology & Implantology, Faculty of Dental Sciences, Sri Ramachandra Institute of Higher Education and Research (SRIHER), Chennai, India fulfilling specific inclusion and exclusion criteria were enrolled in the study after obtaining informed consent. The study protocol was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (IEC/18/DEC/145/51) and was registered in the Clinical Trial Registry of India [Ref No: CTRI/2020/03/0240075].

Participant selection:

Patients of age 21-50 years with moderate to severe periodontitis, sites which had a Probing Pocket Depth (PPD) of ≥ 6 mm and an intrabony component of ≥ 3 mm as detected on Intra oral periapical radiographs (IOPAR) and bone sounding (Sali 2016)¹⁰ were included. Intrabony defect angle of <40 degree (Sali 2016)¹⁰ including Circumferential defect with interdental intrabony defect depth of >3 mm were included. Exclusion Criterias were; Current Smokers (smokers who have smoked $>/=100$ cigarettes in their lifetime and currently smoke) (CDC 2017). Teeth with intrabony defect component and furcation involvement, systemic chronic conditions known to be related to periodontitis and other conditions that can influence systemic inflammation, pregnant / lactating mothers, and previous history of periodontal treatment at the selected site were excluded from the study.

Sample size calculation:

The sample size¹⁰ was calculated with an assumed power of 80% ($\beta = 0.89$) to detect a minimum clinically significant difference in probing depth of 1 mm and a standard deviation of 2.1 and alpha error (5%), a total of 40 (20 per group) patients were required and recruited in the following study. Allowing for dropouts, a total of 50 patients were recruited (25 in each group).

Randomization:

A random number table was used to select 50 subjects for intervention from the target population of 75 and selected subjects were allocated into blocks containing 4 subjects per block. Figure1 shows the CONSORT chart for the present study. In each block, randomisation of the intervention procedures was done using the following code of 1221; 1122; 2112; 1212; 2121, (wherein 1- represents Collagen membrane +Biphasic alloplastic bone substitute (Control) 2- represents Amniotic membrane + Biphasic alloplastic bone substitute (Test)).

Allocation concealment & Blinding:

Allocation concealment was done by placing the interventions in a sealed envelope which was opened by the surgeon prior to start of the surgery. Patients and statistician were blinded to the intervention.

Outcome variables:

The primary outcome variables included the assessment of change in Probing Pocket Depth (PPD)/ Clinical Attachment Level (CAL) and Radiographic bone fill. Secondary outcome variables included Visual Analogue Scale (VAS) (Wong-Baker 1983) & Wound Healing Index (Landry, Turnbull and Howley 1988).

Pre-surgical preparation:

Surgical guides were fabricated with cold cure resin on cast models of patients, obtained by alginate impressions. The guides covered the occlusal surface of the tooth being treated including atleast one tooth mesial and distal to it, extending both buccally and lingually/ palatally. A groove was made in the guide with a fissure bur extending in an apico-coronal direction at the point where probing pocket depth (PPD) and infrabony defect were identified. The groove serves to provide reproducible alignment of the probe (Hu-Friedy PCP-12) during pre-op and post-op measurements. All clinical parameters were analysed by a single trained examiner.

Clinical baseline and 6 month follow-up measurements, Probing Pocket Depth (PPD), Clinical Attachment Loss (CAL), were recorded by taking CEJ as the reference landmark and aligning the probe through the groove in the surgical guide, which ensures proper reproducible angulation.

Each patient involved in the study received a full diagnostic workup, which included Intra -oral periapical radiograph of the affected site, study casts and intraoral photographs. A thorough sub-gingival scaling was performed along with possible elimination of the etiological factors and oral hygiene instructions were given. Routine Blood parameters were checked pre-operatively (FBS/PPBS, BT, CT, CBC, INR). After oral prophylaxis, root planing was done under local anesthesia using Gracey's Area specific curettes. Occlusal adjustments were addressed when indicated. Approximately 3-4 weeks following the nonsurgical periodontal therapy, patients with persistent periodontal pockets (>5mm) were taken up for further periodontal flap surgery.

0.12% of 10ml of chlorhexidine mouth rinse was given to the patient as a preoperative preparation, where the patient was asked to swish for 30 seconds and then expectorated / suctioned to decrease the bacterial load prior to the start of the surgery.

Surgical Intervention:

All surgical procedures were performed by the same expert operator (VL) with a longstanding clinical experience (>10 years) in periodontal surgery.

Under local anesthesia, simplified papilla preservation flap (Coretellini1999)¹¹ was performed. No.63 microsurgical blade was used, the first incision was given across the defect associated papilla in an oblique direction beneath the contact point and was carried out keeping the blade parallel to the long axis of the teeth to avoid excessive thinning of the remaining interdental tissues following which a full thickness mucoperiosteal flap was elevated exposing the intrabony defect. After which, defect debridement and root planing was performed using Gracey's area specific curettes. Root conditioning

was done using Tetracycline Hydrochloride 5% solution and decortication of the defect site was done with the low speed micromotor. The defect was filled with biphasic alloplastic bone substitute material (60% hydroxyapatite: 40% Beta TCP). Following grafting of the defect site, it was followed by placement of Amniotic membrane (Figure2: a-j) or Collagen membrane (Figure3: a-j). The flaps were then trimmed and approximated by external vertical mattress using 4-0 vicryl resorbable sutures and Cyanoacrylate dressing was placed over the surgical site.

Post-surgical care:

Each patient was prescribed analgesics & antibiotics after surgery. Antibiotic Cap. Amoxcillin 500mg thrice daily and analgesic T. Aciclofenac+Paracetamol combination is prescribed twice daily for three days following the surgery. A 60-second rinse with 0.12% CHX is prescribed 3 times/day for first 2 weeks. Patients were recalled at 3, 10 days, 1, 3 & 6 months for professional oral hygiene maintenance and data recording.

Statistical Analysis:

Statistical analysis was performed with SPSS 20 software. Descriptive statistics were recorded as mean values, standard deviations, frequencies and percentages. Analysis was done at patient and technique levels. The primary outcome variable was change in Probing Pocket Depth (PPD) Clinical Attachment Level (CAL) and Radiographic bone fill. Secondary variables included assessment of VAS and WHI scores. The significance of the differences between the groups was evaluated using T-Test. P value <0.005 was considered significant.

Results

Fifty patients (22 males and 28 females) were included in the study. The patients were equally distributed within the AM+BiCP and CM+BiCP groups in terms of demographic characteristics and clinical parameters assessed at baseline (Probing depth/ Clinical Attachment Level and Defect angle) and distribution of the defect walls within AM+BiCP and CM+BiCP (Table1).

The results of the present study showed a mean reduction in the PPD of $2.89 \pm .69$ mm in the CM+BiCP group and $2.95 \pm .57$ mm in the AM+BiCP group and CAL gain of 2.60 ± 1.43 mm in CM+BiCP group 3.18 ± 1.13 mm in the AM+BiCP group at 6 months follow-up with no statistical significance between the groups. In terms of Defect resolution, $98.62 \pm 6.51\%$ was achieved in CM +BiCP group and $98.25 \pm 7.21\%$ in AM +BiCP group (Table 2).

Secondary outcome variables measured were Visual Analog Scale (VAS) and Wound Healing Index (WHI) at Day 3 and Day 10 follow-up. Higher WHI scores and reduced VAS scores were observed in the AM+BiCP group than the CM+BiCP at day 3 and day 10 follow-up, no statistical significance were observed between the two groups (Table 2).

Discussion

The present randomized controlled clinical trial compared clinical/ radiological and patient centered outcomes following the application of Amniotic membrane/Collagen membrane, (porcine derived) in combination with Biphasic Calcium phosphate, (60% Hydroxyapatite: 40% β TCP) for the surgical reconstruction of periodontal intrabony defects.

The two surgical groups were compared in terms of mean differences in Probing Pocket Depth, Clinical Attachment Level and radiographic bone fill at 6 months follow-up. Patient centered outcomes in terms of Visual Analog Scale and Wound Healing Index were assessed at the 3 and 10 day follow-up of the surgical procedure. The types of bony defects included in the present study varied from three-walled to one-walled intrabony defects. It is noteworthy that most defects were also combined. It has been reported that three-walled defects show more predictable results after GTR procedures compared to combined or complex defects. However, the results of the present study revealed that morphologic variations of the bony defects did not influence the efficacy of GTR treatment in either group.

To date, there are no published data on the clinical use and assessment of patient centered outcome measures of AM alone or in conjunction with biphasic alloplastic graft material for the treatment of intrabony defects. The results of the present study showed a mean reduction in the PPD of $2.89 \pm .69$ mm in the CM+BiCP group and $2.95 \pm .57$ mm in the AM+BiCP group and CAL gain of 2.60 ± 1.43 mm in CM+BiCP group 3.18 ± 1.13 mm in the AM+BiCP group at 6 months follow-up with no statistical significance. As evidenced by meta-analysis by Laurell et al., 1998¹² and Parrish LC et al., 2009¹³, the average CAL gain after treating periodontal intrabony defects with biodegradable membranes with and without graft material was reported to be 2.96 mm and 3.50 mm respectively.

Collagen membranes are the commonly employed barrier membranes for GTR, as they facilitate migration of periodontal ligament fibroblasts and provide an early scaffold for neoangiogenesis. However, these types of membranes act mainly as barriers, and they are considered biologically inactive, while owing to the unique characteristics of AM, it acts as a biological membrane, providing wound protection and bacteriostatic effect¹⁴.

As described by Schultz et al¹⁵, dynamic reciprocity is an ongoing, bidirectional interaction among cells and their surrounding microenvironment. These interactions take several forms that may be categorized as direct or indirect. AM offers a scaffold for proliferation and differentiation owing to the presence of elastin, nidogen, collagen types I, III, IV, V, and VI, elastin, and hyaluronic acid. AM contains growth factors such as platelet-derived growth factors alpha and beta and transforming growth factor beta that hasten formation of granulation tissue by stimulating the growth of fibroblasts and stimulates neovascularization¹⁶. Laminin-5 in amniotic membrane has a high affinity to gingival epithelial cells, producing an early physiologic seal with the wound surface and thereby facilitating cell migration, accelerated wound healing¹⁷. AM also contains Fibronectin which acts as the recognition system that guides cell positioning (Hood et al 1977)¹⁸, Fibronectin-cell matrix protein contributes to cell migration

and attachment through the RGD sequence (Ruoslahti et al)¹⁹. One of the major advantages of AM, in comparison to other bio-degradable membranes, is its thickness (320 µm) and good adaptability which owes to the increased adaptability of the membrane to the defect morphology²⁰.

Randomised controlled trial by Shaila et al 2009²¹, Kiany et al 2015²², Sali et al 2016¹⁰ compared AM/Biogide in combination with allograft and xenograft material for the management of grade II furcation defects and intrabony defects. After a post-operative period of 6 and 9 months, both the groups showed significant reduction in PPD, CAL, and percentage of bone fill, without any significant differences between the two groups.

The antiinflammatory, antiinfective and clinical properties of AM were evaluated in 30 patients (Kumar et al 2015)²³, at the end of 24 weeks, increased bone fill and reduced PPD and CAL, and a significant reduction of GCF IL-1β levels and a marginal increase in the hBD-2 levels were observed.

The property of degradability of barrier membrane will influence the surgical outcome of GTR. Porcine-derived collagen membranes in GTR procedures have been shown to resorb within 4 to 6 months²⁴. The degradation period of amniotic membrane can be hypothesised from studies of wound dressing and bladder reconstruction²⁵, it can be put forth that placement of AM as barrier membrane beneath a periodontal flap, prevents the early exposure of the surgical site. The membrane shows reduction in structural stability by 14-21st day as a result of mucoid degeneration^{26,27}. The gains in CAL and reductions in PPD in the present study make it safe to speculate that the absorption of AM was slow enough to produce the desired effects.

In an attempt to further improve the clinical outcomes of GTR, the present study was designed to employ a combined periodontal regenerative technique. The study included one wall, two wall, three wall and combined osseous defects with an intrabony defect angle of $\leq 40^\circ$. Owing to the bioactive properties and chemical similarity to the mineral phase of bone, calcium phosphate based biomaterials (CaP) are widely used for bone regeneration²⁸.

By modifying the HA/β-TCP ratio and, thus, the solubility of ceramic, it is possible to influence the pattern of resorption. On comparison with different ratios of HA and β-TCP (HA100; 80:20; 60:40) in a dog model, the 60:40 group showed more new bone formation and less residual bone mineral remaining at the end of 24 weeks (Ortiz et al 2019²⁹, Puttini I.O,et al 2019³⁰). In the present study we have used Biphasic calcium phosphate in the ratio of 60% HA: 40% β-TCP which would provide the same degree of osteoconductive property in both the intervention groups.

Surgical access to the intrabony defects is selected from three different surgical approaches: the simplified papilla preservation flap; the modified papilla preservation technique; and the crestal incision based on the available interdental space (Cortellini, Tonetti 2015)⁴. The degree of wounding and flap reflection are minimised by these techniques and ensures wound stability, primary closure, and space maintenance (Kao 2015)³¹.

Cortellini 2001³², 2008³³ in multicenter randomized clinical trials, evaluated the outcomes of SPPT to test the generalizability of the added benefits of using barrier membranes in deep intrabony defects along with a variety of regenerative materials. Thus in the present study we have employed the simplified papilla preservation technique to gain access to the periodontal intrabony defects.

Percentage of bone fill at the end six months of the surgical procedure was assessed in terms of Defect resolution. The present study utilised standardised radiography to diminish the variations of the projection geometry between pre- and post-surgical radiographs using the long cone parallel technique with a commercially available positioning guide and the radiographic analysis was done by means of computer aided program. (Image J software). Defect resolution in the present study at 6 months follow-up in CM+BiCP and AM+BiCP groups were $98.62 \pm 6.51\%$ and $98.25 \pm 7.21\%$ respectively with no statistical significance ($p<0.950$).

At present there are no clinical studies evaluating the Patient Centered Outcome measures and wound healing following the Collagen membrane and Amniotic membrane in the management of periodontal intrabony defects. AAP Consensus report on Periodontal regeneration of intrabony defects, 2015³⁴ has concluded that, in future research designs, objective measures of postoperative pain evaluated in real time should be incorporated, inclusion of modulating factors such as treatment modality, operator experience, surgical technique, and patient-related factors. The patient centered outcomes were evaluated using Visual Analog Scale for pain at Day 3 and 10. The mean VAS score at Day 3 were $2.04 \pm .88$ and $2.60 \pm .57$ in the AM+BiCP and CM+BiCP groups respectively with no statistical difference between them ($pvalue < .190$)

Post-operative healing was evaluated using the Laundry's Wound Healing Index. Wound healing after the surgical procedure was assessed (3 days, 10 days) and was quantified and graded. In the present study, the mean WHI scores at day 10 following the surgery were $3.80 \pm .75$ and $4.12 \pm .43$ for CM +BiCP and AM +BiCP groups respectively with no statistical difference ($pvalue < 0.014$). However, in one of the cases in the CM+BiCP group, on day 10 follow-up, there was membrane exposure (with no sign of infection) observed at the surgical site which subsequently healed (following removal of exposed portion of the membrane) allowing for no further complications in regeneration. No post-operative complications were observed during the early healing phase in the AM +BiCP group, owing to the easy adaptability of the amniotic membrane over the defect site providing an early physiologic seal.

A drawback of this study was the use of 2D imaging analysis with the help of Radiovisuography (RVG) instead of 3D analysis with CBCT. Re-entry was not considered, as none of the teeth included in this study were candidates for extraction, a histologic study was not performed.

Guided tissue regeneration in the present era can no longer be considered as a single treatment approach. There is paramount of evidence available to consider GTR as a multifactorial treatment approach comprising careful selection of patients and defects, different surgical techniques, various types of membranes and adjunctive materials and many suturing approaches. All the cited components could be

variously combined to build up different treatment strategies in order to increase the predictability of the treatment outcome.

Conclusion

Within the limitations of the current study, it can be concluded that AM in conjunction with Biphasic calcium phosphate, appears to provide better patient related outcomes and comparable clinical and radiological outcomes as porcine derived Collagen membrane with Biphasic calcium phosphate in the management of periodontal intrabony defects. However future research studies, directed at overcoming the limitations of amniotic membrane which include the handling characteristics, structural stability and limited availability are needed to be performed with long term follow-up.

Abbreviations

GTR – Guided Tissue Regeneration

AM – Amniotic Membrane

CM – Collagen Membrane

CPRT – Combined Periodontal Regenerative Therapy

PPD – Probing Pocket Depth

IOPAR – Intraoral Periapical Radiograph

CDC – Centre For Disease Control

CONSORT – Consolidated Standards of Reporting Trials

CAL – Clinical Attachment Level

WHI – Wound Healing Index

VAS – Visual Analog Scale

FBS – Fasting Blood Sugar

PPBS – Post Prandial Blood Sugar

BT – Bleeding Time

CT – Clotting Time

CBC – Complete Blood Cell Count

INR – International Normalised Ratio

CEJ – Cement Enamel Junction

HA – Hydroxyapatite

β TCP – Beta Tricalcium Phosphate

BiCP – Biphasic Calcium Phosphate

CHX – Chlorhexidine

GCF – Gingival Crevicular Fluid

RGD – Arginine Glycine Aspartate

SPPT – Simplified Papilla Preservation Technique

AAP – American Association Of Periodontology

RVG – Radiovisuography

CBCT – Cone Beam Computer Tomography

2D – Two Dimensional

3D – Three Dimensional

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (IEC/18/DEC/145/51) and was registered in the Clinical Trial Registry of India [Ref No: CTRI/2020/03/0240075].

Consent for publication

Subjects were recruited on obtaining written informed consent.

Availability of data and materials

Available

Competing Interests

No Competing Interests

Funding

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Author's Contributions

NV performed the pre-surgical evaluation and preparation of the study subjects and was a major contributor in writing the manuscript. VL performed the surgical procedures and SKB was involved in the post-op evaluation of the subjects. All authors read and approved the final manuscript.

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Tables

Table 1: Demographic data of the study population with pre-operative clinical parameters (Analysis by t-test square test)

	CM+BiCP (Mean ± Std)	AM+BiCP (Mean ± Std)	p value
AGE (years)	34.84 ±7.13	34.72 ± 6.11	.948
OHI-S	1.60 ± 0.40	1.83 ± 0.48	.083
PPD (mm)	7.36 ± 0.81	7.24 ± 1.01	.611
CAL (mm)	7.32 ± 0.09	7.32 ± 1.02	.500
DEFECT ANGLE (°)	30.02 ± 3.55	31.06 ± 5.17	.237
Distribution of Defect Walls within control and test group			
	One wall	Two wall	Combined defect
CM+BiCP	10 (40.0%)	10(40.0%)	5 (20.0%)
AM+BiCP	11 (44.0%)	8 (32.0%)	6 (24.0%)

Table 2: Intergroup Analysis Of Primary Outcome Variables (PPD,CAL, Defect Resolution) and Secondary

Outcome Variables (VAS & WHI) at day 3 and day 10 follow-up between AM+BiCP and CM+BiCP groups at 6 months follow-up (Analysis by - Student t-test)

	CM+BiCP (Mean ± Std)	AM+BiCP (Mean ± Std)	p value
PRIMARY OUTCOME VARIABLES			
PPD (mm)	2.89±.69	2.95±.57	0.346
CAL (mm)	2.60±1.43	3.18±1.13	0.054
DEFECT RESOLUTION (%)	98.62 ± 6.51 %	98.25 ± 7.21 %	.950
SECONDARY OUTCOME VARIABLES			
DAY 3 WHI	3.36 ± .48	3.44 ± .65	0.313
DAY 10 WHI	3.80 ± .75	4.12 ± .43	0.014
DAY 3 VAS	2.60 ± .57	2.04 ± 0.88	0.190
DAY 10 VAS	1.08 ± 0.75	.92 ± 0.75	0.007

Figures

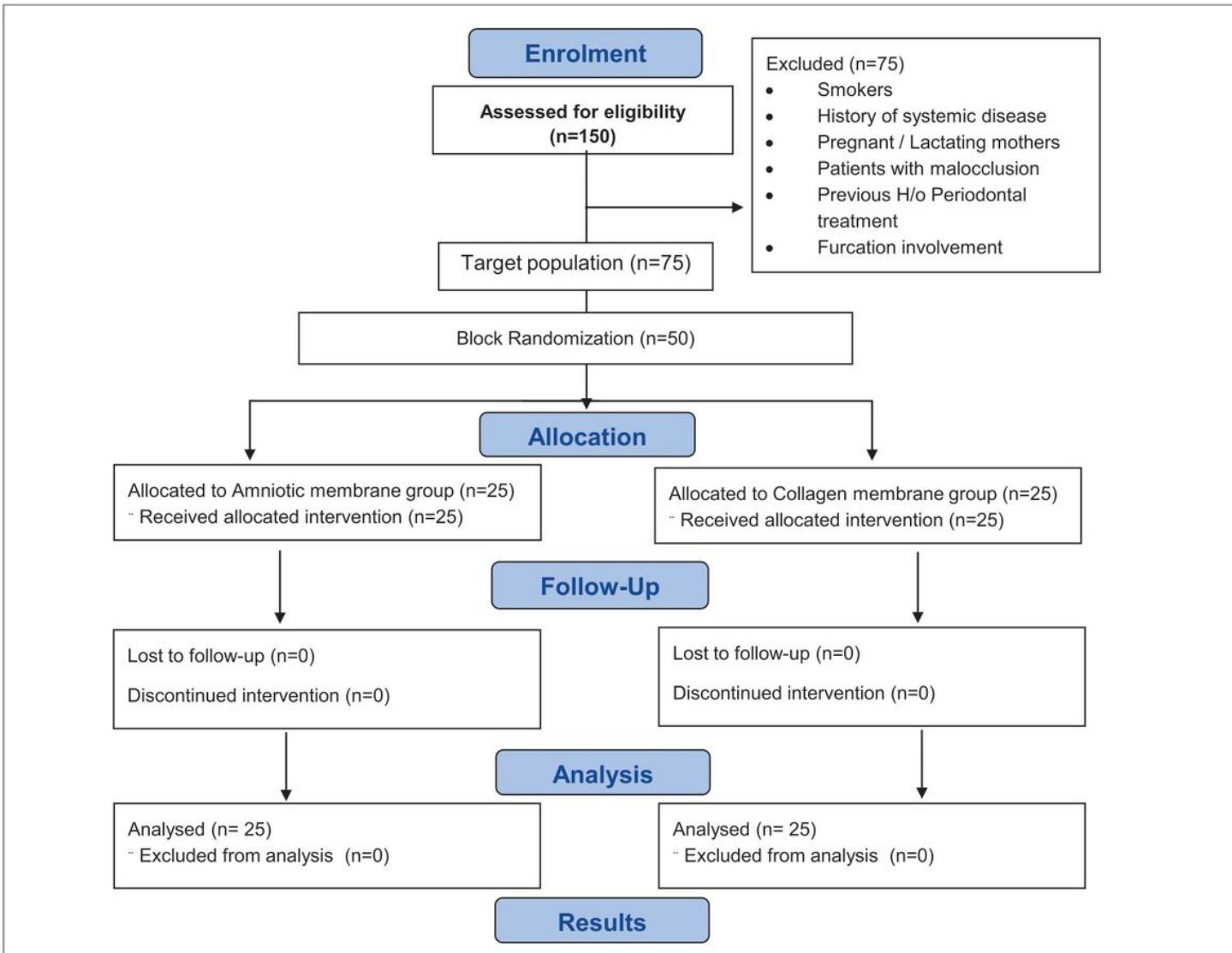


Figure 1

CONSORT flow chart of the present study



Figure 2

(2a) Pre-operative clinical images with acrylic stent in place to measure the PPD, CAL in the region of mesiobuccal [8(9) mm] (2b) Preoperative RVG with intrabony defect in relation to mesial aspect of 26 (2c) Simplified papilla preservation done in relation to mesial of 26 and flaps raised, Intrasurgical clinical measurement of the two wall defect from the CEJ to the base of the defect in relation to mesiobuccal aspect of 26 = 5mm (2d) Root conditioning done with tetracycline in relation to 26 (2e) Defect site grafted with B-ostin HT (HA60%:BTCP40%) (2f) Amniotic membrane placed over the grafted site in relation to mesial aspect of 26 (2g) External vertical mattress suturing done with 4-0 vicryl sutures (2h) Immediate post-operative RVG showing complete defect fill in relation to mesial aspect of 26 (2i) Six months post-operative clinical images with acrylic stent in place to measure the PPD, CAL in the region of mesiobuccal [2(3) mm] (2j) Six months post-operative RVG in relation to mesial aspect of 26 showing complete defect fill



Figure 3

(3a) Pre-operative clinical images with acrylic stent images with acrylic stent in place to measure the PPD, CAL in the region of distobuccal [7(7) mm] aspect of 47 (3b) Preoperative RVG with intrabony defect in relation to distal aspect of 47 (3c) Full thickness flap elevated in relation to 45, 46, 47 with simplified papilla preservation in relation to distal of 47 showing two wall defect from the CEJ to the base of the defect in relation to distobuccal aspect of 47 = 6mm (3d) Root conditioning done with tetracycline in relation to 47 (3e) Defect site grafted with B-ostin HT(HA60%:BTCP40%) and (3f) collagen membrane placed over the grafted area in relation to 47 (3g) External vertical mattress suturing done with 4-0 vicryl sutures (3h) Immediate post-operative RVG showing complete defect fill in relation to distal aspect of 47 (3i) Six months post-operative clinical images with acrylic stent images with acrylic stent in place to measure the PPD, CAL in the region of distobuccal [2(3) mm] aspect of 47 (3j) Six months post-operative RVG in relation to distal aspect of 47 showing complete defect fill