

Title: “Comparative Evaluation of the Effectiveness of Amnioguard (Guided Tissue Regeneration Membrane) in combination with NovaBone Putty and Advanced-Platelet Rich Fibrin (A-PRF Membrane) in combination with NovaBone Putty in the Regeneration of Human Periodontal Infrabony Defects - A Randomized Controlled Clinical Trial.”

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IN

Abstract

Background: When the teeth's attachment system is lost due to periodontal disorders, especially periodontitis, osseous abnormalities, extensive periodontal pockets, and tooth mobility result. Patients experience both practical and aesthetic issues as a result of these impairments. Restoring the periodontal attachment, which includes the cementum, periodontal ligament, and alveolar bone, is the primary objective of periodontal regeneration therapy. Regenerative therapy approaches can be very helpful for infrabony deficiencies, one of the prevalent forms of bone loss brought on by periodontitis.

Objective: 1. To evaluate the effectiveness of AmnioGuard in combination with Nova Bone putty in terms of gain in clinical attachment level(CAL), reduction in probing pocket depth (PPD) and radiographic bone fill in infrabony defects at 6 months post surgery.

2. To evaluate the effectiveness of A-PRF in combination with Nova Bone putty in terms of gain in clinical attachment level, reduction in probing pocket depth and radiographic bone filled 6 months post surgery.

3. To compare the effectiveness of AmnioGuard and A-PRF in combination with Nova Bone putty at 6 months post surgery and bone fill as seen on CBCT.

Methods: Twenty-four infrabony defects in individuals with good systemic health will be treated with NovaBone putty in combination with Amnioguard (Group-A) and NovaBone putty in combination with autologous PRF (Group-B). Clinical and radiographic features will be evaluated before surgery and six months following the procedure. The final data is subjected to statistical analysis. There will be noticeable improvement in both groups' Plaque index, Papillary bleeding index, Periodontal probing depth, and Clinical attachment loss, following periodontal therapy in both groups at 6 months period. On intergroup comparison Amnioguard membrane with NovaBone Putty will show better outcome than A-PRF Membrane with NovaBone Putty.

Results: All the clinical measurements will be recorded at the baseline and 3 months.

Plaque index, which indicates the degree of full mouth supragingival plaque accumulation, will be used to assess the patient's dental hygiene state and will be measured at the one-month follow-up. Although a substantial difference between the groups is not anticipated, Amnioguard + Novabone Putty would show improvement in clinical outcomes, such as CAL and PPD.

Conclusions: Within the constraints of the present study, it will be concluded that Amnioguard membrane with NovaBone Putty will provide additional benefits in the management of chronic periodontitis by promoting better healing and clinical outcomes.

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Original Manuscript

Title: “Comparative Evaluation of the Effectiveness of Amnioguard (Guided Tissue Regeneration Membrane) in combination with NovaBone Putty and Advanced-Platelet Rich Fibrin (A-PRF Membrane) in combination with NovaBone Putty in the Regeneration of Human Periodontal Infrabony Defects – A Randomized Controlled Clinical Trial.”

BACKGROUND: When the teeth's attachment system is lost due to periodontal disorders, especially periodontitis, osseous abnormalities, extensive periodontal pockets, and tooth mobility result. Patients experience both practical and aesthetic issues as a result of these impairments. Restoring the periodontal attachment, which includes the cementum, periodontal ligament, and alveolar bone, is the primary objective of periodontal regeneration therapy. Regenerative therapy approaches can be very helpful for infrabony deficiencies, one of the prevalent forms of bone loss brought on by periodontitis.

AIM: The aim of this study is to compare and evaluate the effectiveness of amnioguard in combination with novabone putty and A-PRF membrane in combination with novabone putty in the regeneration of human periodontal infrabony defects using Cone Beam Computed Tomography(CBCT).

MATERIALS AND METHODS: Twenty-four infrabony defects in individuals with good systemic health will be treated with NovaBone putty in combination with Amnioguard (Group-A) and NovaBone putty in combination with autologous PRF (Group-B). Clinical and radiographic features will be evaluated before surgery and six months following the procedure. The final data is subjected to statistical analysis. There will be noticeable improvement in both groups' Plaque index, Papillary bleeding index, Periodontal probing depth, and Clinical attachment loss, following periodontal therapy in both groups at 6 months period. On intergroup comparison Amnioguard membrane with NovaBone Putty will show better outcome than A-PRF Membrane with NovaBone Putty.

CONCLUSION: Within the constraints of the present study, it will be concluded that

Amnioguard membrane with NovaBone Putty will provide additional benefits in the management of chronic periodontitis by promoting better healing and clinical outcomes.

KEY WORDS: Guided Tissue Regeneration (GTR), Periodontal Osseous Defects, Amnioguard, Novabone Putty, Bioactive Materials, Regenerative Biomaterials

INTRODUCTION

A dysbiotic dental biofilm that gradually destroys bone and periodontal attachment is a hallmark of periodontitis, a chronic, multifactorial, inflammatory illness. Speech, masticatory function, attractiveness, psychological well-being, and quality of life are all negatively impacted by this illness.[1] According to bone resorption patterns, periodontal abnormalities have historically been classified as "supraosseous" (also known as "suprabony") and "intraosseous" (also known as "infrabony"). The position and quantity of osseous walls that remained surrounding the pocket were subsequently used by Goldman and Cohen to categorize infrabony deformities.[2] Regeneration is defined as "the reproduction or reconstitution of a lost or injured part to restore the architecture and function of the periodontium." Restoring the periodontium's structure and functionality is the aim of regenerative periodontal therapy. [3]

Developed from a bioactive glass foundation with additives, Nova Bone Putty is a revolutionary, next-generation calcium phosphorsilicate bone transplant material. In addition to being osteo-stimulative and osteoconductive, this material's main advantages are its enhanced handling and effectiveness. Biocompatible and bioactive, NovaBone Putty is a synthetic alloplastic. It has been demonstrated that Nova Bone putty has hemostatic, anti-inflammatory, and antimicrobial properties. An osteoconductive bioactive substance called Novabone Putty is used to repair osseous defects. It is a premixed mixture of synthetic, absorbable binder and bioactive calcium phosphosilicate particles. Only the elements Ca, P, Na, Si, and O that are naturally present in healthy bone make up the bioactive particle. Glycerine and polyethylene glycol make up the absorbable binder.[4] Amnioguard has a number of advantageous

qualities, including as mechanical strength, flexibility, antifibrotic, anti-inflammatory, antibacterial, and anti-scarring effects. AM's potential to lower inflammation is among its most well-known benefits. It has been demonstrated that AM can function as an antibacterial barrier and lessen bacterial penetration by adhering to the wound surface. Therefore, it has been hypothesized that AM's antibacterial qualities could reduce the likelihood of infection and, depending on the presence of growth factors, aid in the healing process. Because laminin-5 has a strong affinity for gingival epithelial cells, its high concentration throughout AM may hasten the healing process and the membrane's integration with gingival tissue. [5–7] Biological barrier membranes have become more significant in periodontal regeneration in recent years. Since its initial application in skin transplantation in 1910. The leukocytes from A-PRF are particularly effective in influencing the release of growth factors and chemokines. This approach has been found to significantly increase levels of TGF- β 1, EGF, and IGF, as well as enhance the migration and proliferation of human fibroblasts.

OBJECTIVES

1. To evaluate the effectiveness of AmnioGuard in combination with Nova Bone putty in terms of gain in clinical attachment level(CAL), reduction in probing pocket depth (PPD) and radiographic bone fill in infrabony defects at 6 months post surgery.
2. To evaluate the effectiveness of A-PRF in combination with Nova Bone putty in terms of gain in clinical attachment level, reduction in probing pocket depth and radiographic bone filled 6 months post surgery.
3. To compare the effectiveness of AmnioGuard and A-PRF in combination with Nova Bone putty at 6 months post surgery and bone fill as seen on CBCT.

MATERIALS AND METHODS

The purpose and design of the study will be explained to patient and informed consent will be obtained from every patient. The study protocol will be first approved by Ethical committee of Datta Meghe Institute of Higher Education and Research, deemed to be University, Sawangi (Meghe), Wardha.

Recruitment

Participants in this clinical trial will be those receiving treatment at Sharad Pawar Dental College, Datta Meghe Institute of Higher Education and Research's Department of Periodontics and Implantology, or referred for treatment there, and who have periodontal pockets larger than or equal to 4 mm in at least ten permanent teeth. All clinical procedures will be performed there. It is thought that this will enhance trial adherence and recruitment because they will already be receiving treatment.

Inclusion criteria:

- 1) "Presence of at least 1 or 2 radiographically detectable interproximal infrabony osseous defect with probing pocket depth $\geq$ 5 mm and clinical attachment loss $\geq$ 5 mm following initial therapy.
- 2) Depth of intraosseous component of the defect $\geq$ 3 mm by clinical and radiographic means, which will be confirmed on intra-surgical measurement.
- 3) A radiographic base of the defect at least 3 mm coronal to the apex of the tooth.
- 4) Presence of at least 3 mm width of keratinized gingiva around test teeth to allow complete soft tissue coverage of the defect."

Exclusion criteria:

- 1) "Evidence of localized aggressive periodontitis.
- 2) Patients with unacceptable oral hygiene (Plaque Index >1)
- 3) Smokers (recent history of smoking more than 10 cigarettes /day) or who used any of tobacco products.
- 4) Study tooth with inadequate endodontic / restorative treatments.
- 5) Study tooth with mobility exceeding grade II and exhibiting a class III or class IV furcation defect.
- 6) History of periodontal surgical therapy of the selected quadrant.
- 7) Pregnant females or lactating mothers."

MATERIALS:

1. Novabone Putty and Amniogurd

2. Novabone Putty and A-PRF

SAMPLE SIZE ESTIMATIONS:

Mean in Pocket Probing Depth for Aminoguard and Novabone putty (μ_1) Before = 8.6
(As per reference article)

Mean in Pocket Probing Depth for Aminoguard and Novabone putty (μ_2) = After 6.9

-Standard Deviation before: (σ_1) = 1.17

-Standard Deviation After : (σ_2) = 1.10

$Z_{(1-\alpha/2)}$ - Alpha: 0.05 (95% confidence level) = 1.96

$Z_{(1-\beta)}$ - Beta: 0.05 (power = 95%) = 1.64

$$n \geq [(Z_{(1-\alpha/2)} + Z_{(1-\beta)})^2 * (\sigma_1^2 + \sigma_2^2 / r)] / (\mu_1 - \mu_2)^2$$

$$n \geq [(1.96 + 1.64)^2 * (1.17^2 + 1.10^2 / 1)] / (8.6 - 6.9)^2$$

$$= 12$$

Minimum Sample Size Needed for Group 1: 12

Minimum Sample Size Needed for Group 2: 12

Minimum Total Sample Size Needed: 24

RANDOMIZATION

24 participants in which each will receive a different treatment as allocated to Groups 1 and 2. For the participant referring to test group (n=12) NovaBone Putty and amnioguard will be placed into the osseous defect and for control group, osseous defects will be packed with NovaBone Putty and then PRF membrane. The flip coin approach will be used for randomization in order to distribute participants into test and control group. A separate researcher who is not involved in the methods will create the allocation sequence.

PROCEDURE

Participants will be enrolled and randomly assigned to interventions by the researchers in charge of the therapy application. Researchers will undergo training prior to data collection and evaluations, following the procedures and outcome measures outlined.

The UNC-15 probe, which measures plaque index, bleeding on probing, probing depth, pocket depth, and clinical attachment loss, will be used to evaluate participants' periodontal clinical characteristics. Every patient will get lessons on the proper brushing technique as well as a demonstration of good oral hygiene practices.

“After proper examination and diagnosis, initial therapy consisting of oral hygiene instructions, supragingival and subgingival scaling and root planing under local anaesthesia will be performed. Coronoplasty will be performed if necessary. Plaque control instructions will be reinforced until the patient achieved a plaque score of ≤ 1 . A re-evaluation examination will be performed 6 weeks following completion of initial therapy to determine patient's response to the therapy and to confirm the need for periodontal surgery.”

The probe angulations and position will be standardized using a specially designed occlusal acrylic stent. Acrylic will be used to build the occlusal stent on a cast model made from an alginate impression. The occlusal surface of the tooth undergoing treatment, as well as the occlusal surfaces of at least one teeth mesially and distally to the affected tooth, are covered by the occlusal stent. Additionally, the stent will be stretched to cover the teeth's coronal third. To enable repeatable periodontal probe placement, a reference point (slot) will be created on the stent at the deepest location of the affected tooth. The apical border will function as a fixed reference point and be linear.

The patients will be instructed to rinse their mouths for one minute with a 0.2% chlorhexidine gluconate solution prior to the surgical procedure. Only the mouth cavity will be visible due to the patient being draped. We shall maintain asepsis during the entire process. Using a local anesthetic solution of 2% lignocaine with 1:100,000

epinephrine, the surgical site will be anesthetized by either nerve block or infiltration anesthesia, depending on the procedure (Ligno-Ad local anesthetic, Proxim Remedies, India).

Using Bard-Parker number 12 or 15 surgical blades, intracervical (sulcular) incisions will be made on the buccal and lingual portions to begin the conventional procedure, which entails a periodontal access flap. To achieve primary wound closure, the incisions will be carried as far inter-proximally as feasible in order to retain the complete interdental papillae. The flap will include a tooth that is both distal and mesial to the tooth that is linked to the defect. One tooth away from the defect, where further access will be needed, divergent vertical relief incisions will be created if necessary.

Using a periosteal elevator (24G Hu-Friedy, USA), the full-thickness (mucoperiosteal) flap will be mirrored. to make the alveolar bone visible in the osseous defect region. When removing granulomatous tissue from the inner aspect of the flap, great care will be exercised to prevent flap rupture or papillae loss. In order to create autologous PRF, the protocol calls for obtaining 6–10 mL of venous blood from the patient's forearm using an 18 G needle in a sterile plastic container. The blood will then be transferred to new, sterile test tubes without the addition of any anticoagulant, and the initial volume of blood will be split equally between two tubes that are positioned symmetrically around the rotor axis for proper balancing. The tubes will then be centrifuged for 10 minutes at 3000 rpm to create autologous PRF.

Procedure for test group:

"The defect will be completely isolated and hemostasized." To enable quick flap closure following the graft material implantation, the flap will be presutured without tying the knot. With minimal pressure, NovaBone Putty and Amnioguard will be inserted into the test site's osseous defect until the flap is raised, filling it up to the osseous wall's highest coronal level. To ensure primary flap closure, the flap will be coronally adjusted and sutured so that the flap edge is positioned 1 to 2 mm coronal to

CEJ.

Procedure for control group:

"With the exception of packing osseous defects at control sites with NovaBone Putty and covering the bone graft with PRF membrane, the surgical procedure for the control site will be the same as the procedure for the test site."

STATISTICS

The calculation of mean and standard deviation (SD) will be done, and the mean data will be analyzed using a standard statistical method to determine statistical significance. Unpaired and paired t-tests will be employed to evaluate data from the baseline, one month, and three months for each group. A p-value greater than 0.05 will be considered not statistically significant, while a p-value less than 0.05 will be deemed significant.

Evaluation Outcomes

-The primary outcome of this study is to measure probing pocket depth and secondary outcomes will be plaque index, papillary bleeding index, clinical attachment level.

-Probing pocket depth will be measured with the help of acrylic stent and UNC-15 probe. Once the acrylic stent is in place, the UNC-15 probe will be positioned at the appropriate angle to access the deepest part of the interproximal pocket within the crevicular space. The moment the probe makes contact with the stent will be noted in pencil. This method will offer a consistent point of reference.

-Plaque Index will be calculated by index given by Turkesy –Gilmore –Glickman Modification of Quigley-Hein in 1970.

-Papillary Bleeding Index will be calculated by index given Muhlemann H.R.1977

-The Level of Clinical Attachment (CAL) will be measured by UNC-15 probe positioned at base of the pocket.

RESULTS

All the clinical measurements will be recorded at the baseline and 3 months.

Plaque index, which indicates the degree of full mouth supragingival plaque accumulation, will be used to assess the patient's dental hygiene state and will be measured at the one-month follow-up. Although a substantial difference between the groups is not anticipated, Amnioguard + Novabone Putty would show improvement in clinical outcomes, such as CAL and PPD.

DISCUSSION

When periodontal disease results in the loss of the attachment apparatus, regenerative therapy attempts to regenerate periodontal attachment in addition to restoring the original structure and functionality of the periodontal complex, which includes the formation of new cementum on the tooth root and new periodontal attachment between newly formed bone and cementum. [8]

If there are insufficient volumes or dimensions of bone or gingiva for proper function, aesthetics, or prosthetic repair, a dental surgical procedure known as guided tissue regeneration (GTR) uses barrier membranes to direct the development of new bone and gingival tissue. Guided bone regeneration is a frequent term used to describe bone regenerative procedures or ridge augmentation.

Certain cell populations in the periodontium have the ability to form new cementum, alveolar bone, and periodontal ligament if given the opportunity to populate the periodontal wound. [9] These progenitor cell types can be derived from four different sources: 1. Cells of the epithelium

2. Cells produced from gingival connective tissue 3. Cells produced from alveolar bone

4. Cells generated from periodontal ligaments

Uses of this method in practice [10]. Guided tissue regeneration (GTR) is a therapeutic approach that permits the restoration of bone, cementum, and periodontal ligament in degranulated osseous lesions. [11] The definition of "regenerable" has been expanded

by recent developments in periodontal regenerative methods and materials. Recent advancements in periodontal regeneration techniques and materials have broadened the definition of "regenerable" [12]. Since its inception, GTR therapy has been applied in a variety of ways. Non-resorbable occlusal barriers were initially used to identify abnormalities of degranulated periodontal infrabony. Over time, the phrase "combination GTR" was adopted, and flaws were filled with bone and bone replacements before being covered with a barrier. After a while, bioresorbable barriers replaced non-resorbable ones, and biological growth factors have been used more recently to promote recovery.

Alloplastic bone graft materials are commonly employed, and bioactive glass is one of them. The bioactive glass graft material NovaBone® putty has just released. Within minutes of implantation, a special surface reaction happens when bodily fluids come into contact with the bioactive glass substance. The first step is an ionic exchange, in which the material's surface releases cations in return for hydrogen or hydronium ions, creating silanol groups (SiOH). [13] Through a polycondensation reaction, silanol groups attach to one another to generate a silica-rich gel layer on the specific surface. Silica is essential for the development of bioactive glass's bone bonding. A site for the redeposition of calcium and phosphorus from the blood and graft material is created by the silica-rich gel. [14] On top of the silica gel layer, a calcium phosphorus layer develops in a matter of hours. As the thickness and size of the layer increase over time, it transforms into a crystalline layer of hydroxycarbonate apatite (HCA), which is the same as bone material. The foundation for the link between this substance and bone is provided by this apatite layer. [15] The main benefit of bioactive glasses is their quick surface reaction rate, which promotes quick tissue attachment. The surface of the silica-rich layer is negatively charged. This raises the electrostatic charges sufficiently to accelerate the absorption of water. Water molecules and the silanol's hydroxyl groups form hydrogen bonds, which gives bioactive glass its cohesiveness. Osteoblastic stem cells, which develop into osteoblasts and form bone, are drawn to the negatively charged surface of the HCA layer by proteins such growth factors and fibrin, which function as an organic glue. [16] Collagen adheres to the skin and becomes a part of the HCA layer. Collagen expansion up to the junctional epithelium indirectly inhibits

apical migration of the junctional epithelium. Cementum repair may be aided by bioactive glass. [17]

Other characteristics of Amnioguard, including low immunogenicity, pain relief and epithelialization promotion, self-adhesiveness, and aesthetics, have also been demonstrated. The GTR procedure is frequently carried out in conjunction with the insertion of bone grafts or bone graft replacements beneath the membrane. Amniotic membrane (AmnioGuard) has been used in mouth cavity repair [18]. The interconnected porous structure promotes blood vessel formation, cell occlusiveness, and biodegradability. proliferation factors included in AM promote the proliferation of fibroblasts, which speeds up the creation of granulation tissue. [19] Meanwhile, AM promotes neovascularization in the surrounding tissues and vascularizes good granulation tissue.[20] Additionally, AM offers a bioactive matrix enhanced with proteins that promotes cell motility. Therefore, it may be hypothesized that using AM as a GTR membrane could encourage cell migration and wound healing as well as vascularization of the granulation tissue in the defects. The tissue of the amniotic membrane is antibacterial. β -defensins, a significant class of antimicrobial peptides secreted by epithelial cells and a crucial component of the immune system, are produced by amniotic tissue.

A concentrated mixture of growth factors obtained from platelets is known as a platelet concentrate.

Four peripheral blood tubes were taken and put right into a centrifuge that had been preprogrammed.

Centrifugation was carried out using the two procedures listed below: (1) Advanced PRF, sterile plain glass-based vacuum tubes (A-PRF10 tube) (10 mL; 1500 rpm for 14 minutes) and Standard PRF, sterile glass coated plastic tube (9 mL; 2700 rpm for 12 minutes).

Therefore, the present study will be carried out to compare and evaluate effectiveness of NovaBone putty with Amnioguard and NovaBone putty with A-PRF in the treatment of human infrabony defect.

LIMITATIONS

Small sample size: The study might have a limited number of participants, which can impact the generalizability of the results. In smaller studies, the sample may not adequately represent the broader population.

Short Follow-Up Period: If the study follows patients for only a short duration (e.g., 3-6 months), long-term effects of the treatment may not be fully observed.

Single-Centre Study: The study might be conducted at a single institution or clinic (e.g., Sharad Pawar Dental College), which can limit the diversity of the sample. Outcomes may vary in different clinical settings, making it harder to generalize findings to other populations or settings.

Lack of Control Group: If the study does not have a control group (e.g., untreated periodontal defects or treatment with a different material), it becomes difficult to assess whether the observed improvements are truly due to the intervention (Amnioguard or A-PRF with Novabone Putty) or could be attributed to natural healing or other factors.

Variability in Defect Severity: Differences in initial defect severity: The study participants likely had varying degrees of periodontal defects, which could lead to variability in treatment outcomes.

Patient Compliance: Adherence to oral hygiene protocols: The success of periodontal treatment is highly dependent on patient cooperation with post-treatment oral hygiene.

CONCLUSION

The combination of AmnioGuard and NovaBone putty may be more effective in promoting bone regeneration in periodontal osseous defects than the combination of A-PRF and NovaBone putty.

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Not applicable

AUTHOR DECLARATIONS

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects.

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