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Comparative analysis of coronally advanced flap using platelet-rich fibrin membrane and amniotic membrane in gingival recession

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Abstract

Background: Amnion membranes, which originate in the placenta, are rich in stem cells, growth factors, and other proteins and hormones that speed up the body's natural processes for repairing injuries and replacing lost tissue. "It has been established that platelet-rich fibrin (PRF) stimulates bone repair and regeneration by releasing growth factors upon activation from the platelets and being retained inside the fibrin matrix, in addition to stimulating the mitogenic response in the periosteum during normal wound healing. Treatment of localized gingival recession issues was accomplished using a coronally advanced flap (CAF) with either a platelet-rich fibrin (PRF) membrane or a bioresorbable amniotic membrane (AM) in this early controlled, randomized clinical research with an 18-month follow-up."

Materials and Procedures: Sixteen adults in good health were surgically treated for Miller Class I recession abnormalities using CAF in conjunction with either ablative microsurgery (AM) or photorefractive keratectomy (PRK). The plaque index, recession depth, All patients' keratinized gingiva and bleeding gums were evaluated 6 and 18 months after surgery. "The paired t-test, repeated measures analysis of variance, Bonferroni test, and unpaired t-test were used for comparing the two groups, while the other tests were used for analyzing the data within each group."

Results: "At the 6-month and 18-month checkups, there was no discernible difference between the groups on any clinical measure. Comparing gingival recession from baseline to 6 months ($P = 0.000$) and from baseline to 18 months ($P = 0.001$) shows statistical significance." the PRF and amnion groups both showed significant improvements. In contrast, the median value between 6 and 18 months did not reach statistical significance.

Conclusion: In this research, we found that CAF + PRF and CAF + AM both achieve clinically meaningful outcomes in terms of root coverage, with AM demonstrating a greater percentage of root coverage than PRF.

Keywords: Gingival recession, growth factors, periodontal plastic surgery

Introduction

A recession of the gingiva is "the displacement of marginal tissue apical to the cement enamel junction (CEJ) [1]. Root coverage may be attained in a variety of ways; pedicle grafts and soft tissue grafts are only two. The most reliable plastic surgery treatment is the coronally advanced flap (CAF) with sub epithelial connective tissue transplant, which is considered the "gold standard" in periodontal plastic surgery. Better long-term root coverage and remarkable predictability are offset by its scarcity and the much increased morbidity it causes in patients. As a result, allografts provide an excellent chance to conceal gingival recession.

Recently, dentists have begun using placental allografts. "The human placenta is made up of two membranes: the inner amniotic membrane (AM) and the outer chorion membrane. Arthroplasty and other reconstructive operations are only two examples of how AM's processing power has made it more valuable in the medical industry [3]. Cryopreserved AM is useful for periodontal surgery because it promotes cicatrization, wound healing epithelization, migration, and enhanced adhesion. Platelet-derived growth factors have been demonstrated to induce the mitogenic response in the periosteum, allowing for bone repair, during normal wound healing.

It was Ross *et al.* that first made this finding [6]. The regenerative capacity of platelets was originally identified in 1974 [7]. It has been theorized that platelet concentrates, such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), may stimulate the regeneration of periodontal tissue. It has been established that these concentrates speed up the beginning of the wound healing process” [9]. “PRF is a second-generation autologous platelet concentrate with a leukocyte aggregate and a high-density fibrin network, and it mimics the slow polymerization system of growth factors like vascular endothelial growth factor, insulin-like growth factor, platelet-derived growth factor (PDGF), transforming growth factor (TGF), epidermal growth factor (EGF), and basic fibroblast growth factor.” In addition to accelerating hemostasis and wound healing, the special composition of PRF also enhances immunity, cell migration, and proliferation. There is no need for bovine thrombin or an anticoagulant in the preparation of PRF, making it a simple and inexpensive procedure. It can be easily produced, doesn't need any biochemical processing of blood, and shows promise as a regenerating membrane material [10].

Complete recession coverage that is also coordinated with the surrounding tissues is the ultimate goal of recession therapy. It seems to reason that the results obtained with CAF would be improved by the addition of PRF and amnion membrane, both of which promote healing and regeneration.

“To evaluate the efficacy of CAF with a PRF membrane against CAF with a bioresorbable AM in the treatment of localized Miller's Class I gingival recession abnormalities, this pilot randomized clinical trial followed patients for 18 months after therapy.”

Materials and Methods

The clinical trial's protocol was given the go light by the ethics board on April 1st, 2022.

“Patients with Miller's Class I gingival recession who met inclusion and exclusion criteria (sufficient vestibular depth, enough breadth of connected gingiva, and excellent overall health) were recruited from the Outpatient Department of Periodontology. If a patient had at least one Millers Class I recession, appropriate vestibular depth, and adequate breadth of keratinized gingiva, they were regarded to be in excellent systemic health.” Participants who had periodontal procedures within the preceding six months, women who were pregnant or nursing, smokers, and those with poor oral hygiene practices were not included in the study.

At day 0, measurements of the full mouth plaque index were recorded 16 of the patients who were chosen underwent ultrasonic scaling. Patients who kept up their oral hygiene after 4 weeks and had fair ratings for plaque, gingival health, and bleeding on probing index were finally enrolled in the trial. Six individuals—two men and four women—out of the 16 patients (12 men and 4 women) who were chosen and participated in the study were disqualified from the experiment because they neglected to maintain their dental hygiene. Finally, a parallel mouth root coverage investigation was performed on ten male patients at a total of twenty locations (eleven maxillary and nine mandibular). For repeatability of measures to assess the effectiveness of the treatment, an acrylic stent was created at the chosen location. All patients were given thorough information about PRF, the amniotic membrane, and the study's procedures before providing their written informed consent. After that, the volunteers were kept blind and the patients were split into two groups at random using a coin flip. A parallel mouth structure

was used during the experiment. Six and eighteen months following surgery, patients were examined for peri-implantitis, gingival inflammation, bleeding on probing, clinical attachment level, recession depth, recession breadth, attached gingiva width, and gingival thickness. From August 2014 until March 2016, everyone who signed up was followed. The experiment ended within the 18-month window for which data was recalled.

No one in the study knew what was going to happen to them. Patients were given 10 milliliters of a 0.2% chlorhexidine diluted solution to gargle with before surgery. Before commencing surgery, The targeted region was infiltrated with a 2% lignocaine hydrochloride with adrenaline 1:80,000 solution. Root CAF was used to cover all hand-picked recession faults in Miller Class I. There was no cutting of the interdental papillae mesially or distally between the centric eminence junction (CEJ) and a position 1 mm distal to the proximal line angle of the neighboring teeth. On the labial side of the affected tooth, two releasing incisions were made, one horizontal and one vertical, reaching beyond the mucogingival junction. Using BP blade No. 12/15, we produced a subcrestalcrevicular incision at the targeted tooth by combining a horizontal and vertical incision. “Sharp dissection was used to extend a partial-thickness flap apically into the vestibule, enabling the flap to be easily changed as far coronally as necessary, whereas blunt dissection was used to elevate a full-thickness flap 3-4 mm over the marginal bone crest. Connective tissue for the coronally advanced relocated flap was harvested by de-epithelializing the buccal part of the intact papillae. Root planing was performed using universal curettes 2R/2L and/or 4R/4L (Hu-Friedy) on exposed root surfaces.” Both autologous platelet-rich fibrin (PRF) membranes and irradiation, freeze-dried amnion membranes obtained from the tissue bank at Tata Memorial Hospital in Mumbai were used to repair recession abnormalities. Mesial, distal, and proximal surfaces were covered with the membrane after it was trimmed to fit and apical bony recession defects that reached from the CEJ to the neighboring bone. The membrane was covered by coronally repositioning the buccal flap, and non-absorbable 4-0 braided silk was used to hold the CAF in place.

Preparation of fibrin with platelets

Choukroun *et al.*'s (2001) procedure was followed to create the PRF employed in this research. Blood was drawn from the antecubital vein into a sterile, anticoagulant-free tube. Immediately after, the tube was spun for 10 minutes at 3000 rpm (around 400 g). The finished product was centrifuged and then divided into three layers. There was a layer of platelet-poor plasma (PPP) on top, a clot of platelet-rich plasma (PRF) in the centre, and red blood cells (RBCs) in the bottom. Once the PPP was gone, the PRF clot could be readily pried off the RBCs' base and the RBCs were preserved in a thin layer. To make it seem like a membrane, the clot was put on a sterile gauge piece and compressed between two sterile glass slides [9].

Postoperative guidelines

“Extra-oral cold packs and analgesics with anti-inflammatory medicines were used to manage pain and swelling, respectively. Patients were told not to use a toothbrush in the surgical region for four weeks after surgery; mouthwash was recommended instead.” The patients were given instructions on how to prevent the operative area from suffering too much trauma or traction. Patients were checked at 6 months and 18

months after having their sutures removed after 10 days. As a means of comparing therapy efficacy, all obtained data were statistically assessed.

Statistic evaluation

Baseline, six-month, and 18-month post-surgery data were collected and tabulated using statistical methods. New York, Both GraphPad Quick Calcs Software (Online Software 2012, Graph Pad Software Inc., USA) and IBM SPSS (Statistical; Package for the Social Sciences) version 19.0 (USA) were used for the statistical analysis. A paired t-test, an analysis of variance test, and a Bonferroni test for between- and within-group comparisons were performed and an unpaired t-test for contrasting the two groups (Control and experimental).

Results

Table 1: Plaque index mean scores compared between Group A (Control) and Group B (Test).

	Group A (Control group) Mean (SD)	Group B (Test Group) Mean (SD)	Significance
Baseline	2.2	2	p =0.3
6 month	1.1	1.1	p =0.07
18 months	1.2	1.0	p = 0.4
Change from Baseline to 6 month	0.9	0.8	p =0.3
Change from 6 month to 18 months	0.5	0.1	p =0.08
Change from Baseline to 18 months	1.2	1.3	p =0.3

SD = Standard Deviation; NS = Not Significant

Neither group showed a statistically significant difference in values from the beginning to the end of the study period (Table 1). “Degree of the recession (RD): An unpaired t-test was used to compare the extent of the drop in the control and

experimental groups, with a p-value of 0.05 indicating statistical significance, 0.05 indicating no difference, and 0.001 indicating a highly significant difference.”

Table 2: Intergroup comparison of mean recession depth (in mm) between Group A (Control group) and Group B (Test Group)

	Group A (Control group) Mean (SD)	Group B (Test Group) Mean (SD)	Unpaired t-test	Significance
Baseline	2.5	3	t = -1.570	p =0.2
6 month	0.7	0.4	t = 0.607	p =0.7
18 months	0.5	0.2	t = 0.878	p = 0.5

Comparing the width of the keratinized gingiva (KT) between the control and experimental groups, we used an unpaired t-test to determine if there was a significant drop ($p>0.05$), no

difference ($p> 0.05$), or a very significant difference ($p < 0.001$). (Table 3).

Table 3: Intergroup comparison of mean width of keratinized gingiva between Group A (Control group) and Group B (Test Group)

	Group A (Control group) Mean (SD)	Group B (Test Group) Mean (SD)	Significance
Baseline	2	1.5	p =0.01
6 month	3	2.3	p =0.3
18 months	3.2	3.1	p = 0.6
Change from Baseline to 6 month	0.7	1	p =0.2
Change from 6 month to 18 months	0.4	0.7	p =0.4
Change from Baseline to 18 months	1.2	1.2 ±	p =0.2

Discussion

This study compared the efficacy of two treatments for face gingival recessions using a CAF coupled with a PRF membrane and a CAF combined with a bioresorbable AM in a randomized, parallel-mouth controlled experiment. The ultimate goal of periodontal plastic surgery therapies for marginal tissue recession is to regenerate all periodontium-supporting tissues in order to restore aesthetic and functional coverage of the exposed root surface.

To address gingival recession, researchers here used either PRF membrane or AM in a coronally positioned flap operation. Recession defects with enough keratinized gingiva at their apex are ideal candidates for CAF, the recommended

surgical approach. The soft-tissue edge may be reshaped and colored to mix in with the surrounding tissue, and the roots can be successfully covered. Pini Prato *et al.* [11] and Wennström and Zucchelli [12] suggest that this method may result in root coverage of 60-100% of the time on average. However, the keratinized gingiva is not widened and there is little to no impact on periodontal regeneration in cases of gingival recession with this method. Two solutions to CAF were developed: guided tissue regeneration (GTR) and the coronally relocated flap. Root coverage employing autogenous connective tissue in addition to CAF has been shown in many meta-analyses to be the “gold standard” method of root covering without antigenic reaction [12]. Using a CT organ from a donor far away, however, extends the

operation's duration and heightens patient risk. Numerous barrier membranes built on the GTR principle are commercially available to address this problem. With encouraging results, various resorbable allograft membranes for GTR have recently acquired acceptance in clinical settings, and their use has been modified to fit the new idea of biological GTR. Cryopreserved bioresorbable AM, which was employed in this investigation, was helpful in promoting wound healing and epithelization because it promotes gingival cell adhesion, fibroblast development, and angiogenesis, according to Tinti *et al.* [13-16]. Clinical research's mainstay is the development of bioactive surgical additives, which speed up the treatment process. In this regard, PRF seems to be a safe, effective, and natural option with positive outcomes. With varying degrees of efficacy, Gingival recession may be treated by using a membrane made from a patient's own platelet-rich fibrin clot. The [9] PRF developed by Choukroun *et al.* combines many different growth factors developed in France since 2001. This platelet concentrate contains growth factors that govern wound healing. The list of growth factors is likely to be much longer than only platelet-derived growth factor (PDGF) and transforming growth factor (TGF). There was no statistically significant difference in the assessments of plaque, gingival, and bleeding on probing between the two groups. This finding of the current study suggests that patients kept up a high degree of hygiene throughout the investigation. Additionally, the tissue tolerated both the AM and PRF membranes well, with a beautifully integrated coloration and tissue form. There were no statistically significant differences between the groups when comparing the mean values of the differences in pocket probing depth and CAL at baseline. Comparing pocket depth and clinical attachment level from pre-treatment to post-treatment periods of six months, eighteen months, and two years showed no statistically significant differences. These findings corroborate those of a case report by Shetty *et al.* [17] using the same membrane, who also noted complete root coverage and improved gingival biotypes. In this study, gingival recession was considerably decreased ($P = 0.0001$) in the PRF group between baseline and 6 months and between baseline and 18 months. There was no significant change in the mean value between the 6-month and 18-month time periods ($P = 1.000$). These findings are consistent with those of studies by Padma *et al.*, Jankovic *et al.*, Anilkumar *et al.*, and Jankovic *et al.* [18-20], which postulated that a high number of undamaged platelets trapped within a fibrin matrix was responsible for the considerable decrease in gingival recession. The current study's finding that the gingival margin remained stable between 6 and 18 months was consistent with research by Shepherd *et al.* and Gupta *et al.*, who found that when PRP was utilized, the mean recession coverage did not alter between 2 and 4 months after surgery. This may imply that platelet concentrations encourage a more solid attachment to the tooth more quickly. Furthermore, The fibrin matrix of PRF acts as fibrin glue, keeping the flap in place while also stimulating new blood vessel growth and decreasing tissue death. Between baseline and 6 months ($P = 0.000$) and between baseline and 12 months ($P = 0.000$), gingival recession was significantly reduced in the Amnion group. There was no statistically significant difference in the average value across the 6-18 month time span ($P = 0.343$). The findings corroborate the hypotheses of previous research by Mehta *et al.*, Shah *et al.*, Gurinsky, and others, who suggested that processed dehydrated allograft amnion might be an effective substitute for autograft tissue in the treatment of mild to moderate instances of Miller Class I gingival recession. The scientists found that the amnion allograft greatly reduced surgical time and simplified the operation compared to approaches employing autograft or allograft

dermis tissue. Cryopreserved bioresorbable AM was found to be effective in promoting cicatrization and reinforced adhesion in a study of its effects on periodontal soft-tissue healing by Velez *et al.* [4]. Our nonsignificant results between 6 and 18 months suggest that this property provided stable results. However, there was no discernible difference between the groups statistically speaking. Moreover, 18 months after surgery, outcomes were still stable. Consequently, it is hypothesized that AM forms a physiological seal with the host tissue, which limits further bacterial invasion. Moreover, some investigations have shown that amnion may suppress the host immunological response by inhibiting the migration of polymorph nuclear cells in a specific area. In addition to better gingiva coverage, the thin amnion membrane in the Agarwal *et al.* trial allowed for more adaption of the membrane over the recession site [26].

The mean value of the differences between the groups for gingival thickness, gingival breadth, and recession width at all time points was statistically insignificant when compared across groups. These findings imply that PRF and amnion membrane have equivalent therapeutic efficacy. It agrees with the findings of Shetty *et al.* [17] who postulated that the increase in keratinized tissue thickness seen in both groups would assist to keep the clinical outcome steady over time, with less chance of recession recurrence. Shah *et al.* [24] gingival biotype improvement was also indicated six months following treatment with amnion membrane for gingival recession. The soft tissue stability of various periodontal surgical procedures is often better in thick biotypes. Therefore, when it comes to root coverage, amnion allograft has shown to be the most effective option. Comparing CAF + PRF to CAF + bioresorbable AM on gingival recession over a six-month period, recent studies found that the latter was more effective. Results were more consistent at locations treated with bioresorbable AM than at sites treated with PRF. In order to avoid the requirement for a second surgical site and the manufacture of autologous biomaterial, the study results indicate that AM may be used as an additive material in place of subepithelial connective tissue and platelet-rich fibrin (PRF). To verify their long-term stability, more testing is necessary.

Conclusion

The first line of defence against bacterial infection of the tissue is soft tissue upkeep. Although the mechanics and growth factors at play are currently poorly understood, the simplicity with which PRF may be applied in a dental clinic and its positive results, such as reduced bleeding and quick healing, hold hope for future treatments. The biomechanical GTR suggested here, By reducing the likelihood of permanent functional impairment after surgery, bioresorbable AM has been demonstrated to speed recovery, and it also serves as a rich reservoir of stem cells without changing the anatomical and structural makeup of the regenerated tissues. CAF + PRF and CAF + AM were both proven to be similarly effective in delivering clinically significant effects, despite AM showing a higher percentage of root coverage than PRF.

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