COMPARATIVE EVALUATION OF CORONALLY ADVANCED FLAP USING PLATELET-RICH FIBRIN MEMBRANE AND FRESH AMNIOTIC MEMBRANE IN GINGIVAL RECESSION

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ABSTRACT

Background: the aim of this study was to evaluate and compare the effectiveness of root coverage with coronally advanced flap combined with Platelet Rich Fibrin or Fresh Amniotic Membrane.

Materials and Methods: thirty patients had Miller class I or II in maxillary anterior teeth were involved in the present study. Each recession defect was randomly assigned to one of the treating groups: coronally advanced flap with Platelet rich fibrin (PRF group) and coronally advanced flap with fresh amniotic membrane (AM) (AM Group). Clinical measurements of recession depth (RD), root coverage percentage (RC%) pocket depth (PD), clinical attachment level (CAL) and width of keratinized tissue (WKT) were evaluated at the baseline, three months, six months and nine months postoperatively. Statistical analysis was performed for intergroup and intragroup comparisons respectively.

Results: intragroup comparison displayed statistical significant difference with regard to RD and CAL at different time interval. No statistical significant difference between groups at different time interval with regard to RD, PD, CAL while WKT, AM group demonstrated statistical significant increase compared to PRF group.

Conclusion: Both the membranes provided successful and predictable root coverage when combined with CAF. AM was more effective in terms of increase in WKT.

KEY WORDS: Gingival recession; coronally advanced flap; platelet rich fibrin; Fresh amniotic membrane.

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INTRODUCTION

The main objectives of root coverage procedures is to reestablish the gingival margin to the normal position and contour over the root and to obtain an attachment of the tissue over the root surface with the end goal that the gingival sulcus demonstrates minimal depth and no bleeding on probing.[1]

Coronally advanced flap (CAF) was more common technique for root coverage procedure and has varying degree of success. Various regenerative materials and biologic factors are frequently applied with CAF aiming to attain both regeneration of functional attachment apparatus and root coverage. [2]

Platelet-rich fibrin (PRF) has gained prominence status for obtaining periodontal regeneration predictably.[3] PRF obtained using Choukroun's protocol, is a second-generation platelet concentrate. [4] It is considered as an autologous leukocyte and PRF biomaterial that consists of an intimate assemblage of cytokines, structural glycoproteins and glycanic chains enmeshed within a slowly polymerized network of fibrin. As an adjunct to CAF, the beneficial effects of PRF in gingival recession defect coverage has been elucidated with promising results. [5, 6]

Amniotic membrane (AM) or amnion is the placental innermost layer, it is a composite membrane its thickness varies from 0.02 mm to 0.5 mm, [7] consisting of pluripotent cellular element embedded in a semipermeable membranous structure that have three main layers: an avascular mesenchyme made of collagen, a dense basement membrane and a single epithelial stratum. [8]

Over a decade Human amniotic membrane (HAM) has been used successfully a wide range of surgical applications. it has a number of properties that has made its clinical use a success, which includes the lack of human amniotic epithelial cells expression of human leukocyte antigen (HLA) A, BC and DR surface antigens and beta-2-microglobulin which could further contribute to the

lower inflammatory response and lack of rejection phenomenon observed in this type of allografting. [9, 10] Promotion of epithelialization, anti-inflammatory properties, antifibrotic properties, antibacterial properties, and antiangiogenic properties makes amniotic membrane an ideal therapeutic for burns, wound healing, allograft in general surgery for reconstructions, as a scaffold in tissue engineering research and has recently been introduced for periodontal plastic surgery. [11,12,13]

For clinical use, amniotic membrane can be prepared in the following forms: Fresh membrane (hypothermically stored), Dried membrane, Freeze derived irradiated membrane, Stabilized amniotic membrane and Cryopreserved membrane. [14]

Each processing technique of amniotic graft has its advantages and disadvantages. However, the major objective in the graft processing is to maximally remove any risky from the stand points of safe grafting to the patient, but at the same time maintain the natural properties and biological activity of the grafts in order to ensure maximum efficiency and support in wound healing.[15] A fresh hypothermically stored amniotic allograft (HSAM) may improve healing rates by preserving growth factors and living cells, including stem cells, as well as retaining the membrane's native structure.[16]

Based on the biologic properties of PRF and amniotic membrane and its potential, the aim of this study was to evaluate the clinical outcome and the efficacy of coronally advanced flap combined with PRF or fresh amniotic membrane for the treatment of gingival recession

MATERIAL AND METHODS

Study population

This clinical study was conducted on outpatients clinic of the department of oral medicine and periodontology, faculty of dentistry, Suez Canal University, with chief complain of unpleasant

aesthetics and hypersensitivity. All patients were aged between 18-55 year old and did not suffer from systemic illness. The inclusion criteria were presence of Miller Class I or II gingival recessions in maxillary vital anterior teeth or premolars, width of keratinized tissue (WKT) 2 mm, probing depth (PD) <3 mm without bleeding on probing, no radiographic bone loss and the absence of caries or restorations in the areas to be treated. The exclusion criteria involve pregnant or lactating female, heavy smokers and patient with systemic disease (e.g diabetes mellitus, hypertension, cardiac disease and hematologic disease). Each of the subjects that follow inclusion criteria sign informed consent after overall explanation of the nature, benefits and risks of the clinical investigation and surgical procedures.

Sample-size calculation:

The sample size for the study was calculated, and power analysis was done. The power of the study was 95%, with a sample size of 15 recession defects in each group. Overall, 30 patients were convenient to inclusion-exclusion criteria. The patients were randomly allocated (by using lottery method) into 2 group, group I: 15 patients were received CAF + PRF membrane for root coverage and group II: 15 patients were received CAF+ fresh amniotic membrane for root coverage.

Initial Therapy:

Routine radiographic investigations were done for all the selected patients; selected patients had no radiographic bone loss. The patients initially were submitted to a plaque-control program, including oral hygiene instructions to remove habits related to the cause of the recession, scaling, root planning. The patients were instructed to practice a gentle tooth brushing technique using a soft toothbrush.

Clinical parameters

All the clinical parameters data were documented at baseline, 3-months (Ms), 6 months and 9-months postoperatively. To homogenize the reproducibility of clinical data, customized acrylic stent was fabricated. The measurements were performed by Zeffiro William's periodontal probe, the following parameter were recorded at mid-buccal surface of tooth with recession defect: RD measured from cemento-enamel junction (CEJ) to the coronal point of the free margin, Pocket depth (PD), clinical attachement level (CAL) and WKT was measured on the mid-buccal point from the free gingival margin to the mucogingival junction (MGJ).

The percentage (%) of root coverage (RC%) was calculated according to the following formula: "pre-operative recession depth-postoperative recession depth ×100" (preoperative recession depth).

The PRF preparation protocol:

For PRF group, intravenous blood sample was collected in 10-ml centrifuge tubes without antico-agulant and was immediately centrifuged at 3000 rpm for 10 minutes. After centrifugation, the fibrin clot was obtained at the middle layer between the red corpuscle layer formed at the bottom and the supernatant layer formed at the top. The fibrin clot was removed from the tube with sterile tweezers and the RBCs layer that adheres to the fibrin clot was separated with sterile scissors.[17] By squeezing serum out of the PRF clot, a stable fibrin membrane was obtained. [5]

Amniotic membrane:

Fresh AM was delivered from American hospital in Tanta. Under sterile conditions fresh AM was obtained after the elective caesarean delivery from patients who were seronegative (HIV, hepatitis B, C viruses and syphilis). Serological tests were carried out by enzyme linked immune sorbent assay (ELISA). Under a lamellar flow hood, the blood clot was flushed from the placenta with sterile saline. By blunt dissection (through the potential spaces between these two tissues) the inner amniotic membrane was separated from the rest of the chorion and flushed

with sterile saline (2 liters) and later in 4%, 8% and 10% dimethylsulphoxide (DMSO) phosphate buffered saline (PBS) for 5 minutes each, progressively. The grafts were then stored in 50 mg/ml penicillin, 50 mg/ml streptomycin, 100 mg/ml neomycin and 2.5 mg/ml amphotericin B with balanced salt solution at +4ºC. AM is thawed by leaving the vial at room temperature, and then the membrane is transferred to the root surface. [18]

Short-term preservation of AM for research or clinical use and is mainly performed under 0 °C. [19,20,21] Fresh amnion can be maintain in viable conditions up to 6 weeks if properly stored at 4 °C in silver nitrate solution, in 20 % glycerin solution, or in sterile saline after passage through one rinse of 0.025 % sodium hypochlorite solution.[22] Samples preserved fresh, at 4 °C, in 85 % glycerol remain intact for over 1 year.[23]

Surgical procedure:

CAF procedure was performed for patients in both groups according to the technique prescribed by Allen & Miller 1989. [24] before surgery, patients were allowed for rinsing with chlorhexidine mouth wash 0.12%. After obtaining profound anesthesia, initial intrasulcular incisions were made around tooth with gingival recession. From the nearest distal line angle of the most mesial and distal teeth involved the mesiodistal length of the incision was extended and mesial and distal vertical releasing incisions were made in both groups. At least 5 mm apical to the most apical margin of the bony dehiscence a full-thickness flap was then reflected beyond the mucogingival junction. A sharp dissection was performed in the most apical part of the flap to enable passive flap advancement to the CEJ.[5]

The selected membrane was then adjusted and adapted to fully cover the exposed root surface (Fig. 1 & 2 A,B) and sutured with No 4-0 bioresorbable suture (Egysorb). Coronal advancement of the

flap was performed to cover either PRF or fresh amniotic membrane completely and secured in the new position by interdental sutures and vertical releasing incisions on both sides of the flap were then secured with interrupted sutures using No. 4-0 nonabsorbable suture (Egysilk). Periodontal dressing was placed over the surgical area for proper wound stabilization and patient comfort.

Post-Surgical Protocol

Until suture removal (14 days post-surgery), patients were advised to refrain from brushing and flossing around the surgical area. Patients were instructed to use a 0.12% chlorhexidine solution rinse for 1 minute twice daily for 7 days after surgery. Patients received systemic analgesics (Biprofenid capsule 150 mg 3×1) for 3 to 4 days and antibiotics (ciprofloxacin 1 gram vial 2×1) for 4 days and patients were allowed to follow the postoperative oral hygiene measures instructions. All clinical parameters were evaluated at the followup visits postoperatively on 3 Ms (Figs. 1C and 2C), 6 Ms (Figs. 1D and 2D) and 9 Ms (Figs 1E and 2E).

Statistical analysis

Data was analyzed using Statistical Package for Social Science software computer program version 23 (SPSS, Inc., Chicago, IL, USA). Quantitative parametric data was presented in mean and standard deviation while quantitative non-parametric data was presented in median (MD) and range (R) (minimum-maximum) Student's t-test (unpaired) was used to compare between two different groups of parametric data while Mann whitney U was used to compare between two different groups of non-parametric. Repeated measures ANOVA (Analysis of variance) was used for comparing more than two related groups of parametric data while Friedman was used for comparing more than two related groups of non-parametric. P value less than 0.05 was considered statistically significant.
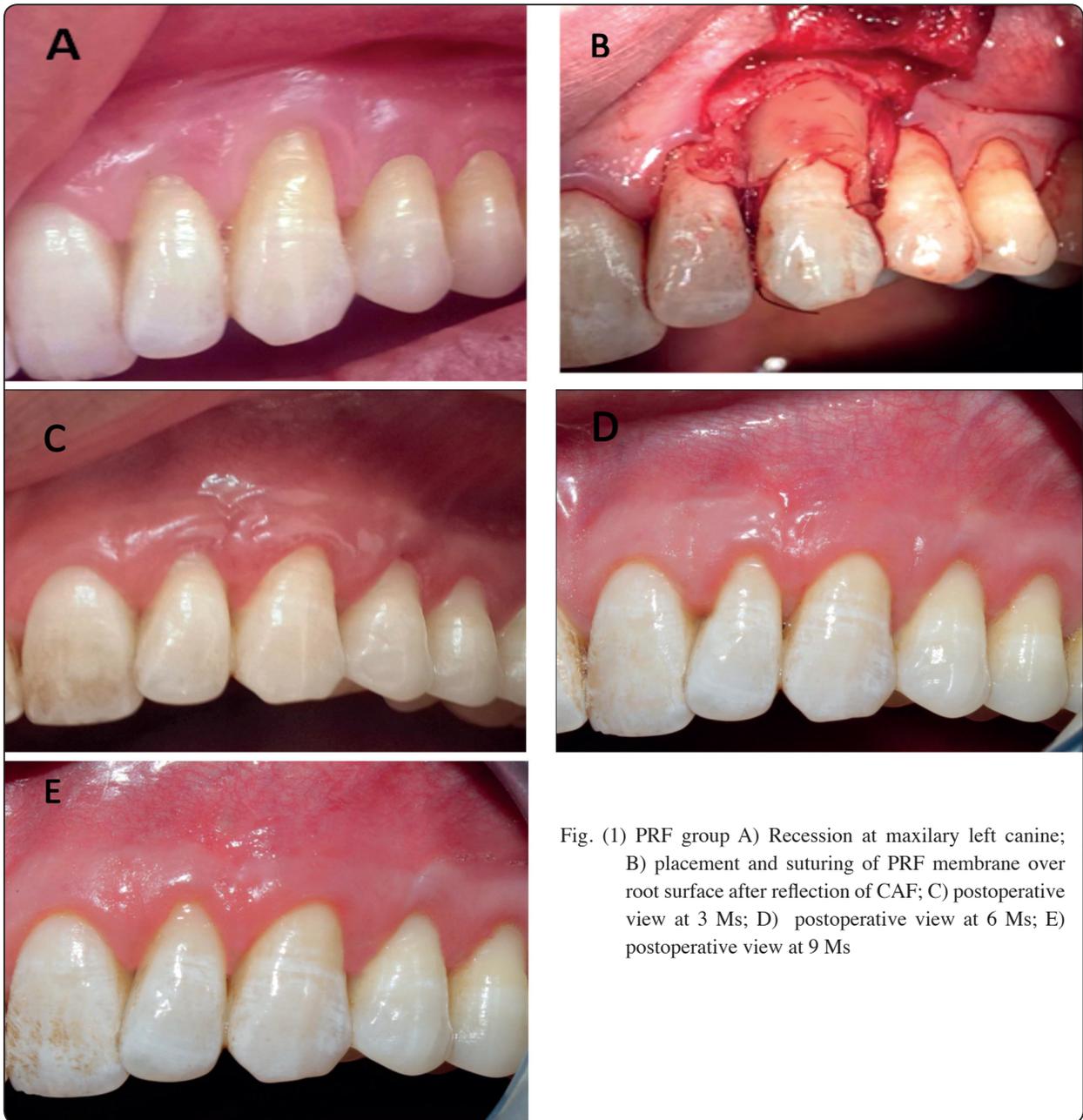
Fig. (1) PRF group A) Recession at maxilary left canine; B) placement and suturing of PRF membrane over root surface after reflection of CAF; C) postoperative view at 3 Ms; D) postoperative view at 6 Ms; E) postoperative view at 9 Ms

Fig. (2) AM group A) Recession at maxilary right canine; B) placement and suturing of AM membrane over root surface after reflection of CAF; C) postoperative view at 3 Ms; D) postoperative view at 6 Ms; E) postoperative view at 9 Ms
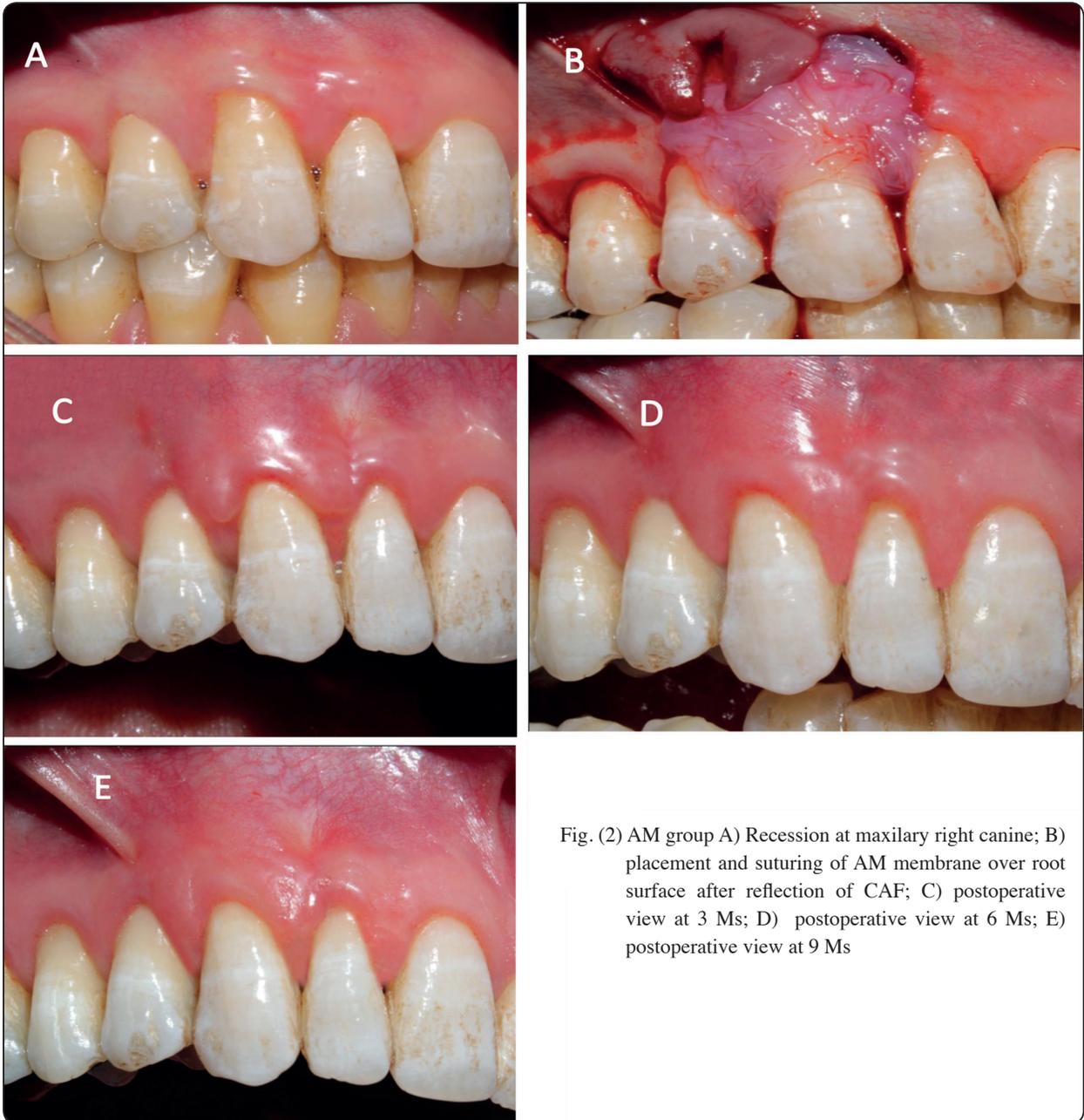
TABLE (1): Showed intragroup and intergroup comparison of RD at different time interval

	PRF group		AM group		Pg
	Median	Range	Median	Range	
Basal	3.00	2.00-4.00	4.00	2.00-4.00	0.11
3M	1.00	.00-2.00	1.00	.00-2.00	1.00
6M	1.00	.00-2.00	1.00	.00-2.00	0.8
9M	1.00	.00-3.00	2.00	.00-2.00	0.93
Pt	<0.001*		<0.001*		
Post-hoc	P1=<0.001*, <0.001*, <0.001*, <0.001* P2=0.8 , 0.3 P3=0.4		P1=<0.001*, <0.001*, <0.001* P2=0.67 , 0.39 P3=0.67		

*P:Probability *:significance <0.05*
Pg : significance for comparison between PRF & AM groups(Test used: Mann whitney)
Pt : significance for comparison between Basal ,3M ,6M and 9M within each group (Test used: Friedman followed by pairwise comparions)
P1: significance relative to Basal (with 3M, 6M, 9M), P2: significance relative to 3Ml (with 6M, 9M)
P3: significance relative to 6Ml (with 9M)

TABLE (2): Showed intragroup and intergroup comparison of RC% at different time interval

	PRF group		AM group		Pg
	Mean	SD	Mean	SD	
3M	68.60%	±24.21%	70.93%	±22.79%	0.78
6M	65.27%	±22.99%	67.60%	±23.83%	0.78
9M	57.00%	±27.88%	64.27%	±24.34%	0.45
Pt	0.07		0.13		
Pt	<0.001*		<0.001*		
Post-hoc	P1=<0.001*, <0.001*, <0.001*, <0.001* P2=0.8 , 0.3 P3=0.4		P1=<0.001*, <0.001*, <0.001* P2=0.67 , 0.39 P3=0.67		

Data expressed as mean±SD   SD: standard deviation
*P:Probability *:significance <0.05   Pg : significance for comparison between PRF & AM groups (Test used: Student's t-test) Pt : significance for comparison between Basal ,3M ,6M and 9M within each group (Test used: repeated measures ANOVA)*

TABLE (3): Showed intragroup and intergroup comparison of PD at different time interval

	PRF group		AM group		Pg
	MD	Range	MD	Range	
Basal	1.00	1.00-2.00	2.00	1.00-2.00	0.2
3M	3.00	2.00-3.00	3.00	2.00-3.00	0.2
6M	2.00	1.00-3.00	2.00	2.00-3.00	0.3
9M	2.00	1.00-3.00	2.00	1.00-2.00	0.5
Pt	<0.001*		<0.001*		
Post-hoc	P1=0.001*, 0.2, 0.4 P2=0.028* , 0.009* P3=0.67		P1=<0.001*, 0.12, 0.5 P2=0.01* , 0.001* P3=0.35		

*P:Probability *:significance <0.05 Pg : significance for comparison between PRF & AM groups(Test used: Mann whitney) Pt : significance for comparison between Basal , 3M , 6M and 9M within each group (Test used: Friedman followed by pairwise comparionss) P1: significance relative to Basal (with 3M,6M,9M) P2: significance relative to 3Ml(with 6M,9M) P3: significance relative to 6Ml (with 9M)*

TABLE (4): Showed intragroup and intergroup comparison of CAL at different time interval

	PRF group		AM group		Pg
	Median	Range	Median	Range	
Basal	5.00	4.00-6.00	5.00	4.00-6.00	0.56
3M	4.00	1.00-5.00	4.00	2.00-5.00	0.96
6M	3.00	1.00-5.00	3.00	1.00-5.00	0.9
9M	3.00	1.00-5.00	3.00	1.00-5.00	0.87
Pt	<0.001*		<0.001*		
Post-hoc	P1=0.024*, 0.002*, 0.001* P2=0.39 , 0.25 P3=0.77		P1=0.009*, <0.001*, <0.001* P2=0.1 , 0.07 P3=0.8		

*P: Probability *:significance <0.05   Pg : significance for comparison between PRF & AM groups (Test used: Mann whitney) Pt : significance for comparison between Basal , 3M , 6M and 9M within each group (Test used: Friedman followed by pairwise comparionss) P1: significance relative to Basal (with 3M, 6M,9M), P2: significance relative to 3Ml (with 6M,9M), P3: significance relative to 6Ml(with 9M)*

TABLE (5): Showed intragroup and intergroup comparison of WKT at different time interval

	PRF group		AM group		P^g
	Median	Range	Median	Range	
Basal	3.00	2.00-4.00	3.00	2.00-4.00	0.46
3M	3.00	3.00-4.00	4.00	3.00-5.00	0.05
6M	3.00	3.00-4.00	4.00	3.00-5.00	0.006*
9M	3.00	3.00-4.00	4.00	3.00-5.00	0.02*
P^t	0.003*		<0.001*		
Post-hoc	P1=0.016*, 0.056, 0.056 P2=0.6 , 0.6 P3=1.00		P1=0.001*, <0.001*, 0.001* P2=0.8 , 0.88 P3=0.7		

*P:Probability   *:significance <0.05*

Pg : significance for comparison between PRF & AM groups (Test used: Mann whitney)

Pt : significance for comparison between Basal ,3M ,6M and 9M within each group(Test used: Friedman followed by pairwise comparionss)

P1: significance relative to Basal(with 3M,6M,9M), P2: significance relative to 3Ml(with 6M,9M)

P3: significance relative to 6Ml(with 9M),

RESULTS

All surgical procedures were tolerated well by the subjects and no postoperative complications were observed.

For PRF group MD (R) for RD (table 1) were statistically significant decrease (P ≤0.001) at 3Ms, 6Ms and 9 Ms compared to baseline while intragroup comparison of mean of RC% (table 2) showed no statistical significant difference (P>0.05) at 3 MS (68.6 %), 6 Ms (65.7%), and 9 Ms (65.7%). With regard to PD (table 3) there was statistical significant increase (P ≤0.001) at 3 Ms that statistically insignificant decrease at 6 Ms and 9 Ms compared to baseline the changes in RD and PD were accompanied with statistical significant decrease (P ≤0.001) in MD (R) of CAL at 3 Ms, 6

Ms and 9 Ms compared to baseline. WKT (table 5) presented statistical significant increase (P≤0.05) at 3 Ms compared to baseline but showed no statistical significant difference (P>0.05) at 6 Ms and 9 Ms compared to baseline.

For AM group RD (table 1) MD (R) was statistically significant decrease (P ≤0.001) at 3 Ms, 6 Ms and 9 Ms compared to baseline. No statistical significant difference (P>0.05) of the means of RC% (table 2) at 3 Ms 70.9 %, 6 Ms (67.6 %) and 9 Ms (64.27%). PD (table 3) displayed statistical significant increase (P≤ 0.001) at 3 Ms, that statistically insignificant (P>0.05) decrease at 6 Ms and 9 Ms compared to baseline. Improvement in MD (R) of CAL (table 4) was observed at all time interval, where it was statistically significant decrease (P ≤0.001) at 3 Ms, 6 Ms and 9 Ms compared to its MD (R) at baseline. Intragroup comparison of MD(R) of WKT (table 5) presented statistically significant increase at 3 Ms, 6 Ms and 9 Ms compared to its MD(R) at baseline.

Intergroup comparisons produced no statistical significant difference between groups with regard to RD, RC%, PD and CAL at different time interval but AM group showed statistical significant increase (P≤0.05) in WKT compared to PRF at 6 Ms and 9Ms postoperative time interval.

DISCUSSION

Primary reason for the root coverage procedures was the high esthetic needs and increasing awareness of patient. In order to increase the efficacy of root coverage procedures, reduce the morbidity of the technique, and improve clinical outcomes, PRF and Amniotic membrane was applied with CAF in the present study and the effectiveness of both membranes in treatment of gingival recession was compared.

In the present study both PRF and AM were effectively reduce RD at different time interval. Although AM presented more preferable results in

RD than PRF where RC% in AM group was 70.9 %, 67.6 % and 64.27% at 3 Ms, 6 Ms and 9 Ms respectively while PRF showed RC % (68.6 %), (65.7%) and (57%) at 3 Ms, 6 Ms and 9 Ms, this difference were not statistically significant.

The fibrin matrix itself within PRF has biologic functions and mechanical adhesive properties closely resemble fibringlue where it preserve the flap in stable and high position, promote neoangiogenesis, decreases shrinkage and necrosis of the flap thus maximal root covering was guaranteed by stabilization and remodeling of the gingival flap in the highest possible covering position.[25] These may explain the findings of the present study with regard to RD where PRF group showed statistical significant decrease in RD from baseline at 3 Ms where RC% was (69.80%), at 6 Ms where RC% was (64.80%) and at 9 Ms where RC% was (63.13%). Similarily, in a study of Jankovic et al.,[5] PRF significantly reduces RD with mean RC% (75%). Moreover, Murugan 2015[26] concluded that treatment of isolated gingival recession with CAF combined with PRF resulted in reduction of RD with percentage of RC 74.16% at 6 Ms. The difference in the results may be attributed to different baseline data. Also studies of Reddy S et al.,[27] Padma et al.,[3] and Tunali et al.,[28] showed gingival recession treated with PRF resulted in enhanced root coverage. In contrast to the our results, a systematic review and meta-analysis of Moraschini et al.,[29] documented that rapid degradation of PRF membrane interfere with early stabilization of the flap during healing which results in insignificant decrease in RD when applied in root coverage procedure.

The presence of vascular growth factor and induction of fibroblast proliferation within AM results in more creep attachment and better healing due to prevention of necrosis of terminal portion of the flap and acceleration of tissue maturation and angiogenesis.[30] Accordingly, AM in the present study showed statistical significant decrease in RD

at 3 Ms where RC% was (69.27%), at 6 Ms where RC% was (65.93%) and at 9 Ms where RC% was (65.93%). This was in accordance with the study of George et al.,[7] where the amniotic membrane results in significant reduction in RD compared to CAF alone. Also, in Esteves et al.'s[31] study, the outcome of AM when used for root coverage results in significant reduction of RD from 3.14 ± 1.24 to 2.76 ± 1.00 (RC%22). Similarly AnkitA et al.,[6] concluded that both AM and PRF showed reduction in RD with RC% (24% and 22%) respectively when used for root coverage procedure.

The present study showed preferable reduction of RD than aforementioned studies which may be attributed to the fresh amniotic membrane that used in the present study which are folded several times over the root surface, well adapted over root surface and improved clinical handling compared to dried AM. It has been reported previously that methods of preparation and processing of AM reduces cellular viability and the selective elution of soluble proteins [32] Moreover, it affects the angiogenic factor profile of the AM. [33]

Different studies showing promising results of using Am in oral and maxillofacial surgery where it accelerated healing and regeneration. Experimental oronasal fistulas of 15 mm diameter were created in minipig model and 14 days later closed by different soft tissue substitutes. Complete closure of the palate in two of three animals in the group experiencing reconstruction by a multi-layered xenogeneic HAM whereas the cases closed by a dermal regeneration template showed increased and inflamed fistulas at 40 days after surgery.[34] More jaw movement were encountered when inserting fresh AM to the articular fossa after the induction of joint ankylosis in 24 rabbits, and microscopic examination showed no fibrous adhesions. [35]

With regard to PD and CAL no statistical significant difference between PRF and AM group at all-time interval. In PRF group there

were statistically significant increase (P≤0.001) in PD at 3 Ms that statistically significant decreased (P≥0.05) at 6 Ms and 9 Ms compared to baseline. There was statistical significant gain in CAL at 3 Ms (P≤0.02), 6 Ms (P≤0.002) and 9 Ms (P≤0.001) compare to baseline. These may attributed to the incorporation of circulating platelets, leukocyte, stem cells within fibrin matrix constituents of PRF [36] also cytokines gradually released during fibrin-matrix remodeling which may responsible for the clinically observed healing properties of PRF.[2] These results were in agreement to results observed by Jankovic *et al.*,[5] Padma *et al.*, [3] and Uraz *et al.*,[37] they concluded that PRF with CAF showed statistical significant gain in CAL.

In AM group PD also statistically significant increased (P≤ 0.001) at 3 months that statistically significant decrease at 6 Ms (p≥ 0.12) and 9 Ms (P≥0.5) also there was statistical significant decrease at 3 Ms (P≤ 0.009), at 6 Ms and 9 Ms (P≤ 0.001) compared to baseline. Similarly Sumit et al., [38] reported that gingival recession class I treated with AM combined with CAF resulted in gain in CAL. These finding may be attributed to the close similarity between AM and the oral mucosal basement membrane and AM have several types of laminins, which can enhance regeneration, hasten tissue adhesion, facilitate angiogenesis and tissues preservation, all of which enhance healing of periodontal tissues and might result in reduction in PPD and decrease in CAL.[39]

With regard to WKT, PRF statistically significant increase (P≤ 0.05) the WKT at 3 Ms compared to baseline but WKT showed no statistical significant change (P≥ 0.05) at 6 Ms and 9 Ms compared to baseline, these findings was in accordance with the study of Keceli et al.,[40] where PRF showed no improvement in WKT when used for treatment of Miller's Class-I and II gingival recessions, This may be due to mucogingival Junction Junction

(MGJ) natural tendency to return into its genetically predetermined localization has a wide variation and takes long time which may change the amount of WKT significantly. Conversely, studies of Eren G et al., [41] and Tunali et al., [28] had shown that PRF increased WKT same as the gold standard SCTG.

In the other side AM showed statistical significant increase (P≤0.001) at 3 Ms, 6 Ms and 9 Ms. Moreover, the increase in the WKT in AM group was statistically significant compared to PRF group at 6 Ms (P≤ 0.001) and 9 Ms (P≤ 0.05). These finding was in agreement of Ghahroudi et al., [42] in a study and in a case report of Aravind et al.,[43] have found increase in WKT with AM when compared to CTG. These may be explained by the presence of mitogenic factors, such as cytokeratins, vimentin, keratinocyte growth factor and intracellular cytoskeletal filaments, present in the placental membrane which might aid in maintenance of the position of mucogingival junction enhance epithelial cells keratinization.[7]

From the present study it was concluded that, both the materials; PRF and AM proved to be equally effective materials in terms of recession coverage, but amniotic membrane provide more better result in term of increased WKT and no adverse reactions during the course of the present study. However, the fact that amniotic membrane is a biological product raises a number of questions. Intra- and inter-donor variations make it a non-standardized product and that certainly affects its performance, also, the processing and preservation method used and the storage time can also alter the amniotic membrane composition and further influence its biological properties and its clinical effect.[44] More clinical trials and researches on different types of recession defects are necessary to identify the potential of AM as guided tissue regeneration also further researches are required to identify its potential for guided bone regeneration.

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