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A Comparison Of The Effect Of Concentrated Growth Factor And Platelet Rich Fibrin In The Treatment Of Multiple Gingival Recession Defects – A Randomized Split Mouth Clinical Study

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ABSTRACT

Aim: The aim of the study was to compare the effect of Platelet Rich Fibrin (PRF) and Concentrated Growth Factor (CGF) in the treatment of bilateral Miller's class II multiple gingival recession defects.

MATERIALS AND METHODS: Fifteen patients with bilateral multiple Miller's class II gingival recession defectswere selected for this study and were divided into two groups using split mouth design. Fifteen sites treated with CGF (Group A) and 15 sites were treated with PRF (Group B), using pouch and tunnel technique. Clinical parameters such asgingival recession depth (GRD), gingival recession width (GRW), probing pocket depth (PPD), clinical attachment level (CAL), keratinized gingiva width (KTW), gingival thickness (GT) and mean root coverage (MRC) were included in this study and the treatment effectivenesswas determined at 90^{th} and 180^{th} day after the surgical procedures.

RESULTS: There was a statistically significant reduction in GRD, GRW, PPD, KTW, GT, and gain in CAL from 0 to 180^{th} day in Group A compared to Group B.There was also statistically significant mean root coverage in Group A compared to Group B.

CONCLUSION: It can be concluded that both PRF and CGF along with tunnel technique is a successful alternative for effective treatment of multiple gingival recession defects.

1. Introduction

Gingival recession is a mucogingival condition where there is exposure of the root surface caused by the apical migration of gingival margin from the cementoenamel junction (CEJ). It may be localized or generalized, and one needs to have a thorough understanding about the etiology to treat them successfully. Chan et al categorised the etiological factors of gingival recession into precipitating factors and predisposing factors. The predisposing factors include bone dehiscence, tooth malposition, inadequate keratinized tissue, aberrant frenum and precipitating factors include traumatic tooth brushing, plaque induced inflammation, orthodontic treatment, subgingival restorations, and habit such as smoking.[1] The consequences of gingival recession are esthetic concerns, plaque retention, hypersensitivity and root caries.[2]A variety of surgical techniques have been proposed for covering the exposed root surface which include the pedicle grafts such as coronally or laterally advanced flaps, free gingival grafts, and subepithelial connective tissue grafts.[3]Wide range of non-resorbable

and absorbable barrier membranes have been used for better regeneration of the lost periodontal tissues.

Periodontal regeneration is a complex multi-factorial process involving biologic events like cell adhesion, migration, proliferation, and differentiation.[4]Various new regenerative materials have been tried, one such material is autologous platelet concentrates which contains growth factors (GFs) that control the process of wound healing and have a critical role in cell migration, cell proliferation and angiogenesis for tissue re-generation.[5]Platelet rich fibrin (PRF) described by **Choukroun et al**is an autologous second-generation platelet concentrate system.[6] PRF holds onto the growth factors entangled in the fibrin network resulting in their sustained release over a period that can accelerate the wound healing process. The prepared PRF is found to be rich in fibronectin and vitronectin proteins.[7]Concentrated growth factor (CGF) is a platelet concentrate, first developed by **Sacco** in **2006** that contains platelets that are concentrated in a gel layer containing fibrin matrix as same as PRF.[8]CGF contains larger, denser, and richer GFs in the fibrin matrix that will give better regenerative capacity and high versatility as its fibrin clot have higher rate of cohesion which provides protection from plasmin degradation and results in higher fibrin tensile strength and stability.[9] In the past very limited comparative studies have been done using CGF and PRF in the treatment of gingival recession. Hence the aim of the study was to compare the effect of Platelet Rich Fibrin (PRF) and Concentrated Growth Factor (CGF) in the treatment of bilateral Miller's class II multiple gingival recession defects.

2. Materials and Methods

2.1 Study Design

The subjects for this study were selected from the outpatient Department of Periodontology, Meenakshi Ammal Dental College and Hospital, Chennai. The study sample consisted of 15 systemically healthy subjects, both males and females, with bilateral Miller's class II multiple gingival recession defects in the anterior and premolar region of maxilla or mandible. 30 sites from 15 subjects were randomly allocated by coin toss method into two groups (Group A and B) in a split mouth design, based on inclusion and exclusion criteria.Group A(Test Site)comprised of 15 sites with Miller's class II multiple gingival recession defects treated with CGF using pouch and tunnel technique and Group B (Control Site) comprised of15 sites with Miller's class II multiple gingival recession defects treated with PRF using pouch and tunnel technique. Ethical clearance for the study was obtained from Institutional Review Board of Meenakshi Ammal Dental College, Maduravoyal, Chennai-600 095 (IRB No: MADC/IRB-XXV/2018/394). The study was explained to the patients and a written informed consent was obtained from those who agreed to voluntarily participate in this study.

2.2 Inclusion and Exclusion Criteria

Inclusion criteria for the selected subjects includes: patients above 18 years of age, bilateral Miller's class II multiple gingival recession defects in the anterior or premolar region of upper and lower quadrant, gingival recession depth (GRD) > 2mm, gingival thickness (GT) > 1mm, keratinized gingiva width (KGW) < 2mm, probing pocket depth (PPD) < 3mm and

patients who maintain good oral hygiene. Exclusion criteria includes: recession defects associated with demineralization / caries, deep abrasion or restoration and teeth with evidence of pulpal pathology, evidence of radiographic interproximal bone loss, history of any periodontal surgery in the defect area within past 1 year, trauma from occlusion, usage of medications that interfere with wound healing, pregnant females and lactating mothers, systemically compromised patients, alcoholics and smokers.

2.3 Clinical Parameters Assessed

Periodontal parameters such as recession depth, recession width, probing pocket depth, clinical attachment level, keratinized gingiva width, gingival thickness and mean root coverage were recorded using Williams periodontal probe to the nearest millimetre.

2.4Surgical phase:

All the periodontal parameters were assessed prior to the surgery. Each patient was prepared for surgery with an initial phase of therapy, which included, oral prophylaxis, root planing and oral hygiene instructions. The patients were reassessed after 7 days for their oral hygiene compliance. At the time of surgery intravenous blood of 10ml was collected from each subject who were enrolled for the study to procure CGF and PRF for test and control site, respectively.

2.4.1 CGF preparation:

Intravenous blood was collected in 10ml test tube without adding anticoagulant solutions. The tube was immediately centrifuged in CGF centrifuge machine[#] at 30 seconds acceleration, 2700 rpm for 2min, 2400 rpm for 4 min, 2700 rpm for 4 min, 3000 rpm for 3 min and 36 seconds deceleration and stopped. At the end of centrifugation, there were four blood fractions: 1) The upper serum layer 2) The second buffy coat layer 3) Growth factor and unipotent stem cell layer (CGF) 4) Lower red blood cell layer. The CGF clot was removed and squeezed to get a CGF membrane with a thickness of 1mm.[8]

2.4.2 PRF preparation:

Intravenous blood sample was taken without adding anticoagulant in 10ml test tubes which was then immediately centrifuged at 3000 rpm for 10 minutes. A fibrin clot was obtained in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top. The clot was obtained and squeezed to get PRF membrane.[6]

2.4.3 Surgical Procedure:

The povidone-iodine solution of 7.5% was used to cleanse the peri-oral area. The surgical area to be treated was then anesthetized with adequate amount of local anesthesia using 2% lignocaine hydrochloride containing 1:80,000 epinephrine. After local anesthetic administration and appropriate anesthesia of the surgical site, with the help of No.15 blade, a sulcular incision was given through each recession area. Without giving an interdental

incision the tissues were gradually undermined including the base of interdental papilla without involving the tip of interdental papilla. A full thickness mucoperiosteal flap was extended apically above the mucogingival junction preparing a tunnel using a tunnelling instrument so that there is no flap tension and also favours coronal advancing of the flap as well as placement of the graft. CGF membrane was manipulated into the pouch and through the tunnel to cover the recipient site in group A (Fig 1) and PRF membrane was placed in group B (Fig 2). Then the facial enamel surface of each tooth to be sutured is acid etched with 37% phosphoric acid for less than 5 seconds, thoroughly washed and dried. Then with the use of 4-0 black silk suture, the flap is coronally advanced by placing the horizontal mattress suture approximately 2-3mm apical to the gingival margin of each tooth to be sutured. The flap was advanced coronally to the most coronal level without any flap tension. The suture was finally positioned at the midcoronal point of each tooth and bonded to the tooth surface with the use of light cure composite resin. During the initial stages of healing, this can effectively prevent apical relapse of gingival margin. Coe-pakwas placed.

Postoperative instructions and medications (amoxycillin 500mg 3 times a day for 5 days and aceclofenac 100 mg 3 times a day for 3 days) were given with instructions to rinse the mouth daily with a solution of 0.2% chlorhexidinedigluconate for 7 days after the surgery. The patients were recalled after 10 days for periodontal dressing and suture removal. Maintenance schedule following suture removal at 90^{th} and 180^{th} day for measuring clinical parameters post-operatively.

2.5 Statistical Analysis

Statistical analysis of the data was done using IBM SPSS Statistics, version 26 software package. Descriptive statistics including mean and standard deviation were calculated for various clinical parameters. Normality of the data was assessed using Kolmogorov-Smirnov test. Further analysis was done using non-parametric tests since the data significantly deviate from normal distribution. The mean rank values at baseline, 3 and 6 months were compared using Friedman test. The differences between the test and control group were compared using Mann-Whitney test. Statistical significance was kept at $p < 0.05$. Comparison between two matched pairs were done using Wilcoxon Signed Ranks Test.

2.6 Results

There was statistically significant reduction in recession depth, recession width as well as gain in clinical attachment level within the groups from0 to 180^{th} day. On comparison between the groups there was better reduction in probing pocket depth, recession depth, recession width as well asgain in clinical attachment level in Group A from 0 to 90^{th} day and 0 to 180^{th} day than Group B (Table 1, Table 2, Table 3, Table 4).

There was statistically significant increase in keratinized tissuewidth and gingival thickness within the groups when observed from0 to 180^{th} day. On comparison between the groups there was an increase in gingival thickness from 0 to 90^{th} day and 0 to 180^{th} day which was

statistically significant in both Group A than Group B.There was statistically significant mean root coverage at 180th dayin Group A compared to Group B (Table 5, Table 6, Table 7).

2.7 Discussion

Demand for esthetic dental treatments have been increasing rapidly over the past few decades. Various attempts are made to cover the denuded root surface to overcome the problems caused by gingival recession. Advanced techniques and regenerative materials help in enhancing the tissue quality resulting in better treatment outcome. Tunnelling technique is efficient in treating multiple recession defects as they do not involve the interdental papillawhich makes it a minimally invasive procedure.[10]**Cieslik-Wegemund M et al** reported that maintaining intact papilla without vertical incision, can prevent keloid formation in the surgical site after wound healing.[11] Eventhough, CTG is considered to be the gold standard,[12] PRF and CGF which are two different platelet concentrates, can be used as a successful alternative as a soft tissue graft keeping the patient comfortable and painless at the time of surgery as it does not require two surgical sites. An experimental study done by **Sacco et al** reported that CGF contained more growth factors than PRF and has a more rigid fibrinogen structure.[13] **Bozkurt Dogan S et al** used CGF as a barrier membrane along with CAF technique for multiple gingival recession, and reported that it resulted in enhanced tissue healing and increased width of attached gingiva.[14] In the present study, coronally anchored suturing technique using light cure composite resin was done as described in the study done by **Zadeh**which will help in placement of gingival margin more coronal to CEJ to compensate for the post-treatment tissue shrinkage and to achieve complete root coverage after surgical procedure.[15]

The results of mean probing pocket depth in group A from 0 day to 180th day was statistically significant and in contrast to the study done by **Akcan et al** where probing depth was not statistically significantly when compared between the CGF group and connective tissue graft (CTG) group at six months in which Miller's class I gingival recession (Table 3).[16]The mean probing pocket depth from 0 day to 180th day in group B was not statistically significant and was in contrast with the study done by **Aroca et al** in whichthe clinical effects ofmodified coronally advanced flap (MCAF) plus PRF grouphad significant improvement in probing pocket depth when compared to MCAF alone group at six months post-treatment(Table 3).[17]

The mean clinical attachment level in group A from 0 day to 180th day, there was a gain in clinical attachment level which was statistically significant and was in accordance with the study done by **Akcan et al**(Table 4).[16]A statistically significant difference was also found in group B correlating with the study done by **Padma et al** [18] which showed a significant enhancement in the clinical attachment level(Table 4).

In group A there was a reduction in the gingival recession depth when observed from 0 day to 180th day, which was found to be statistically significant and was in accordance with **Akcan et al**[17]who reported reduction in gingival recession depth six months post-treatment when compared with baseline data (Table 1). The reduction ingingival recession depth in group B

was statistically significant when the mean change was analysed from 0 day to 180th day, which was in accordance with the study done by **MuruganThamaraiselvan et al** (Table 1).[19]However, the present study was in contrast to the study done by **Santosh Gupta et al**[20] where the reduction in gingival recession depth was not found to be significant. The significance in gingival recession depth reduction in the present study may be due to the superior and sustained release of growth factors from the platelet concentrates such as CGF and PRF which helps in cell proliferation and angiogenesis as reported by

Elisa Borsani et al.[21]
The mean gingival recession width in Group A from 0 day to 180th day was statistically significant and was in correlation with the study done by **Akcan et al**(Table 2).[16]The mean gingival recession width in Group B from 0 day was also reduced significantly during 90th day and 180th day and was in accordance with the study done by **Aroca et al**[17]which reported a statistically significant gain in recession width at 180th day post treatment in the PRF group(Table 2).

The study done by **Akcan et al**[16]showedsignificant result in increasing the keratinized gingiva width similar to the present study in which group A showed statistically significant improvement in 'keratinized gingiva width' from 0 to 180th day(Table 5). The statistically significant difference of 'keratinized gingiva width' in group B from 0 day to 180th day was in accordance with the study done by **SamedKuka et al.**[22]However this was in contrast to the study done by **Aroca et al**[17]. The treatment with platelet concentrates increases the keratinized gingiva width due to the release of growth factors which helps in the stimulation and proliferation of gingival and periodontal fibroblasts. The gain in keratinized tissue width in our study may also be due to the tunnel technique which provides adequate blood supply to the graft underneath, that can help in progressive coronal improvement of the gingival margin over time which was disclosed in a study done by **Tavelli et al**.[23]

On comparing the mean gingival thickness, thechange from 0 day to 180th day was statistically significantin both group A and group B(Table 6).One of the main advantages of the present study is the use of tunnel technique which has no vertical releasing incisions or involves the interdental papilla so as to provide a scaffold effect beneath the flap where the graft is secured, which may help to promote wound healing withfavorable gingival thickening, as reported by **Rebele et al**.[24]

The key function of all fibrin clots is the sustained release of growth factors which attributes to tissue regeneration. The present study used CGF and PRF as a barrier membrane that facilitate wound healing and formation of new attachment. The degradation rate of CGF was stated to be slower than other fibrin clots that may be due to the combined effect of initial and late phase of growth factor release as reported in a study done by **Isobe et al** which may haveenabled the improvement in the clinical parameters post-operatively.[25]On the other hand, **Roman F et al** reported that incubation of PRF of equine origin established interrupted platelet cytoplasm with peripheral emigration of organelles which indicated the deterioration

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of PRF membrane at a short time point.[26]The above mentioned studies enlightens the use of two different platelet concentrates depending on the purpose of treatment.

The mean root coverage at 180th day for Group A was 95.55±11.72% and for Group B was 70.00±16.90% which was statistically significant and in accordance with the study done by **Dogan et al**where CGF group had MRC of 86.67% which may be due to creeping attachment, increasing KTW and GT, resulting in better attachment gain (Table 7).[14]The mean root coverage percentage for Group B was less when compared with Group A, which was in contrast with the study done by **SantoshGupta et al**[20], where the MRC was 91±19.98%, at 6-months post-treatment for PRF group(Table 7). The subjective observation and collected data over a timeline have demonstrated the usefulness of platelet concentrates and tunnel technique for the treatment of gingival recession.

Table 1: Comparison of mean, standard deviation and test of significance of Depth of Gingival Recession (GRD) within and between the groups at different time intervals

Time interval	Group A (Mean±SD in mm)	Group B (Mean±SD in mm)	P- Value (Between groups)
0th day	2.47±0.51	2.73±0.59	0.218(NS)
90th day	0.13±0.35	0.47±0.64	0.095(NS)
180th day	0.13±0.35	0.87±0.51	0.000(S)
Mean change 0 to 90th day	2.34±0.16	2.26±0.05	0.000(S)
p-value	0.000(S)	0.000(S)	
Mean change 0 to 180th day	2.34±0.16	1.86±0.08	0.000(S)
p-value	0.000(S)	0.000(S)	
Mean change 90th to 180th day		0.4±0.13	0.014(S)
p-value	1.000(NS)	0.014(S)	

S - Statistically significant | NS - Statistically not significant
Level of Significance p < 0.0 5

Table 2: Comparison of mean, standard deviation and test of significance of Width of Gingival Recession (GRW) within and between the groups at different time intervals

Time interval	Group A (Mean±SD in mm)	Group B (Mean±SD in mm)	P- Value (Between groups)
0th day	3.60±0.98	3.67±1.04	0.891(NS)
90th day	0.27±0.70	0.67±0.97	0.135(NS)
180th day	0.27±0.70	1.2±0.86	0.002(S)
Mean change 0 to 90th day	3.33±0.28	3.00±0.07	0.000(S)
p-value	0.000(S)	0.001(S)	
Mean change 0 to 180th day	3.33±0.28	2.47±0.18	0.000(S)
p-value	0.000(S)	0.001(S)	
Mean change 90th to 180th day	0	0.53±0.11	0.023(S)
p-value	1.000(NS)	0.023(S)	

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S - Statistically significant | NS - Statistically not significant
Level of Significance p < 0.0 5

Table 3: Comparison of mean, standard deviation and test of significance of probing pocket depth (PPD) within and between the groups at different time intervals

Time interval	Group A (Mean±SD in mm)	Group B (Mean±SD in mm)	P- Value (Between groups)
0th day	1.87±0.35	1.80±0.56	0.63(NS)
90th day	1.20±0.41	1.20±0.41	1.00(NS)
180th day	1.33±0.48	1.53±0.51	0.27(NS)
Mean change 0 to 90th day	0.67±0.06	0.6±0.15	0.000(S)
p-value	0.002(S)	0.003(S)	
Mean change 0 to 180th day	0.54±0.13	0.27±0.05	0.001(S)
p-value	0.005(S)	0.102(NS)	
Mean change 90th to 180th day	0.13±0.07	0.33±0.1	0.008(S)
p-value	0.157(NS)	0.025(S)	

S - Statistically significant | NS - Statistically not significant
Level of Significance p < 0.0 5

Table 4: Comparison of mean, standard deviation and test of significance of Clinical Attachment Level (CAL) within and between the groups at different time intervals

Time interval	Group A (Mean±SD in mm)	Group B (Mean±SD in mm)	P- Value (Between groups)
0th day	4.33±0.72	4.53±0.64	0.430(NS)
90th day	1.27±0.70	1.67±0.72	0.118(NS)
180th day	1.47±0.99	2.40±0.63	0.003(S)
Mean change 0 to 90th day	3.06±0.02	2.86±0.08	0.000(S)
p-value	0.001(S)	0.000(S)	
Mean change 0 to 180th day	2.86±0.27	2.13±0.01	0.000(S)
p-value	0.001(S)	0.000(S)	
Mean change 90th to 180th day	0.2±0.29	0.73±0.09	0.000(S)
p-value	0.083(NS)	0.002(S)	

S - Statistically significant | NS - Statistically not significant|Level of Significance p < 0.0 5

Table 5: Comparison of mean, standard deviation and test of significance of Width of Keratinized Tissue (KTW) within and between the groups at different time intervals

Time interval	Group A (Mean±SD in mm)	Group B (Mean±SD in mm)	P- Value (Between groups)
0th day	2.00±0.00	2.00±0.00	1.000(NS)

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90th day	4.13±0.51	3.80±0.77	0.205(NS)
180th day	4.13±0.51	3.40±0.63	0.003(S)
Mean change 0 to 90th day	2.13±0.51	1.80±0.77	0.000(S)
p-value	0.000(S)	0.001(S)	
Mean change 0 to 180th day	2.13±0.51	1.40±0.63	0.000(S)
p-value	0.000(S)	0.001(S)	
Mean change 90th to 180th day	0	0.40±0.14	0.014(S)
p-value	1.000(NS)	0.014(S)	

S - Statistically significant | NS - Statistically not significant |Level of Significance p < 0.0 5

Table 6: Comparison of mean, standard deviation and test of significance of Gingival Thickness (GT) within and between the groups at different time intervals

Time interval	**Group A (Mean±SD in mm)**	**Group B (Mean±SD in mm)**	**P- Value (Between groups)**
0th day	1.40±0.50	1.33±0.48	0.710(NS)
90th day	2.53±0.51	2.00±0.53	0.013(S)
180th day	2.53±0.51	2.00±0.53	0.013(S)
Mean change 0 to 90th day	1.13±0.01	0.67±0.05	0.000(S)
p-value	0.000(S)	0.002(S)	
Mean change 0 to 180th day	1.13±0.01	0.67±0.05	0.000(S)
p-value	0.000(S)	0.002(S)	
Mean change 90th to 180th day	0	0	1.000(NS)
p-value	1.000(NS)	1.000(NS)	

S - Statistically significant | NS - Statistically not significant
Level of Significance p < 0.0 5

Table 7: Comparison of Mean root coverage between the groups

Group A (Mean±SD)	**Group B (Mean±SD)**	**P- Value (Between groups)**
95.55±11.72	70.00±16.90	0.000(S)

Fig. 1

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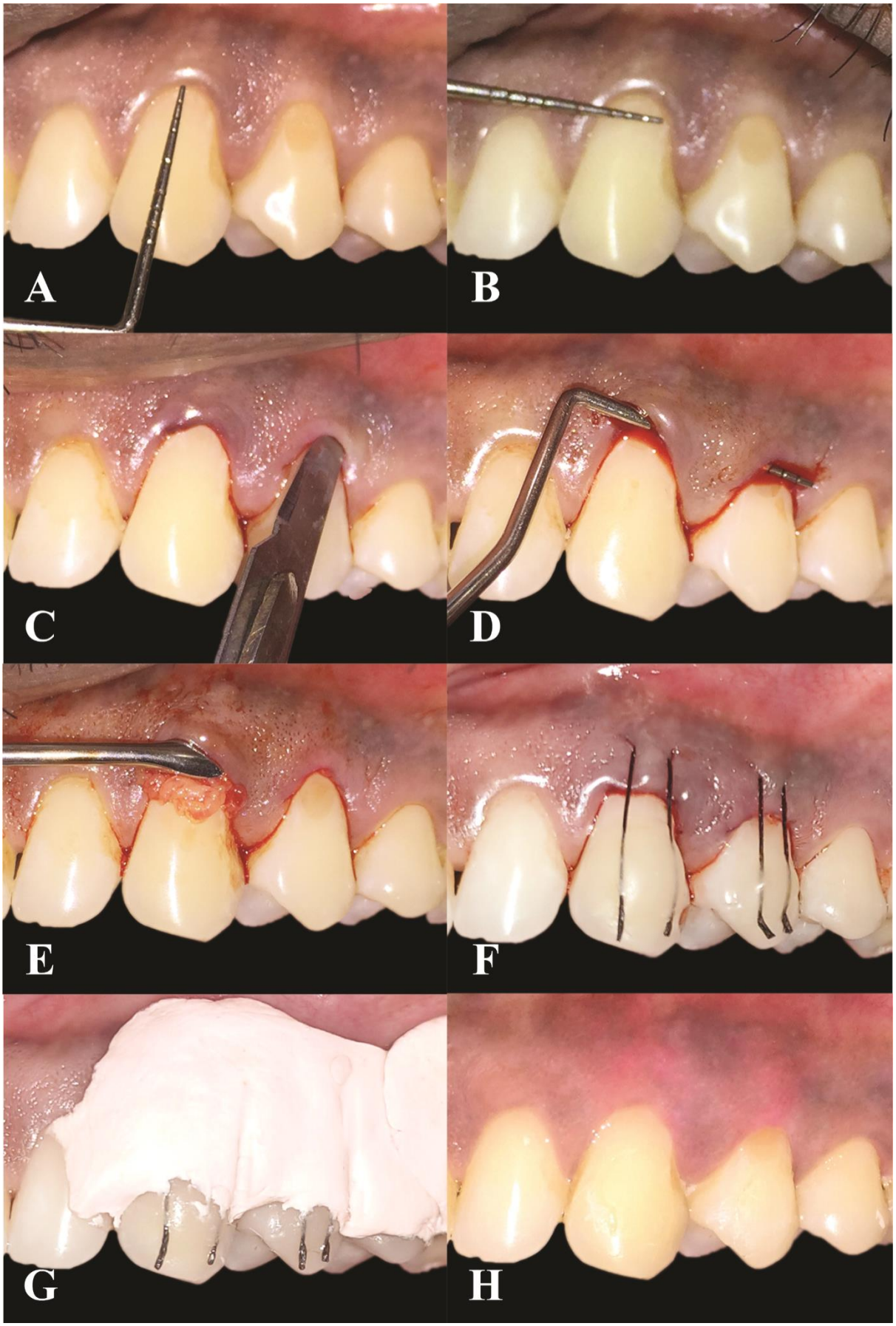

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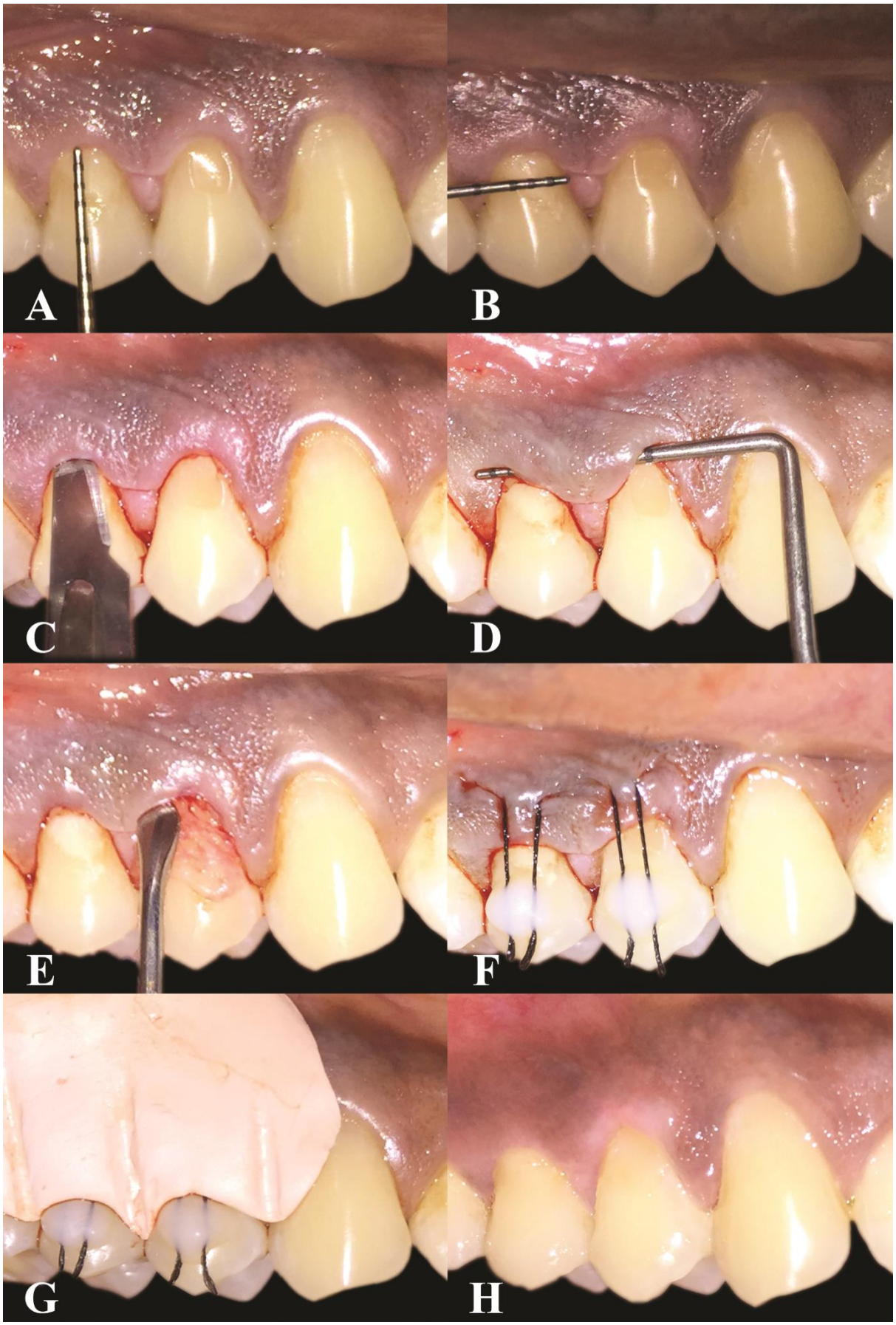
Fig 2.



CONCLUSION

CGF and PRF are autologous membranes with no adverse reactions, economical and readily prepared. This stdy showed improvement in the recession depth, recession width, keratinized tissue width, gingival thickness and gain in clinical attachment level in both the study groups.To the best of our knowledge, this is the first randomized control study which has compared the effectiveness of CGF and PRF using tunnel technique in treating gingival recession. These results of the present study must be validated for their effectiveness by conducting studies with larger sample size and long-term follow-up.

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