

Concentrated growth factor in the treatment of adjacent multiple gingival recessions: a split-mouth randomized clinical trial

Şeyma Bozkurt Doğan¹, Figen Öngöz Dede¹, Umut Ballı¹, Elif N. Atalay¹ and Mustafa C. Durmuşlar²

¹Department of Periodontology, Faculty of Dentistry, Bülent Ecevit University, Zonguldak, Turkey; ²Department of Oral Maxillofacial Surgery, Faculty of Dentistry, Bülent Ecevit University, Zonguldak, Turkey

Bozkurt Doğan Ş, Öngöz Dede F, Ballı U, Atalay EN, Durmuşlar MC. Concentrated growth factor in the treatment of adjacent multiple gingival recessions: a split-mouth randomized clinical trial. *J Clin Periodontol* 2015; 42: 868–875. doi: 10.1111/jcpe.12444.

Abstract

Aim: The aim of this study was to determine the clinical effect of concentrated growth factor (CGF) in combination with coronally advanced flap (CAF) compared to CAF alone for the treatment of multiple adjacent gingival recessions (GRs).

Materials and Methods: Twenty patients with a total of 119 Miller Class I and II GRs in the maxilla were included to this study. Recessions were randomly treated according to a split-mouth design by means of CAF + CGF (test; 60 defects) or CAF (control; 59 defects). Clinical outcomes were evaluated at baseline and 6 months after surgery.

Results: The mean root coverage (MRC) was 82.06% and 86.67%, complete root coverage (CRC) was 45.8% (27/59) and 56.7% (34/60) for CAF and CAF + CGF, respectively at 6th month. Statistically no difference was demonstrated between the two groups in terms of recession depth (RD), MRC and CRC at 6th month. The increase in width of keratinized gingiva (KGW) and gingival thickness (GT) were statistically significant in the CAF + CGF group compared to the CAF group at 6th month.

Conclusions: The use of CGF in combination with CAF did not provide additional benefits in RD, CRC and MRC. This study suggests that use of CGF + CAF may increase the success of GRs because of a significant increase in KGW and GT.

Key words: concentrated growth factor; gingival recession; growth factor; plastic surgery; platelet-rich fibrin; root coverage

Accepted for publication 10 August 2015

Conflict of interest and source of funding statement

The authors declare that they have no conflicts of interest related to this study.

This study was financially supported by the Bülent Ecevit University Research Grant (2013-62550515-03).

The gingival recessions (GRs) have been successfully treated by several periodontal plastic surgery procedures. The ultimate goal of these plastic periodontal surgical procedures is the coverage of exposed root surface and an optimal aesthetic outcome (Aroca et al. 2009, Nieri et al. 2013). The main indications for root

coverage (RC) procedures are aesthetic concern, root hypersensitivity, prevention or management of root caries and cervical abrasion, enhancement of restorative outcomes and facilitation of plaque control efforts (Chambrone & Tatakis 2015). Numerous periodontal plastic surgical procedures have been performed

in the treatment of GR. Among the different types of procedures used, coronally advanced flap (CAF) is the most frequent approach, and when combined with a connective tissue graft (CTG) is accepted as a gold standard therapy (Aroca et al. 2009, Graziani et al. 2014).

The most recent advances in RC were focused on the adjunctive agents of platelet concentrates (PCs) as a way to accelerate wound healing and repair. Five major growth factors (GFs), platelet-derived GF, fibroblast GF, transforming GF-beta and insulin-like GF-I were released from the local application of PCs, which may enable better tissue re-generation and healing (Luo et al. 2015). These GFs are mainly located in the blood plasma and platelets (Clark 2001). So platelets have been widely used to accelerate tissue re-generation and repair in dental and medical area. As first generation of PC, platelet-rich plasma (PRP) is identified as one mediator that has many GFs. Recently, investigators introduced platelet-rich fibrin (PRF) and concentrated growth factors (CGF). CGF was first developed by Sacco (Sohn et al. 2011). CGF is produced by the centrifugation of venous blood and platelets are concentrated in a gel layer containing fibrin matrix as same as PRF (Rodella et al. 2011). However, a different centrifugation speed permits the isolation of much larger, denser and richer GFs in fibrin matrix from CGF (Sohn et al. 2009). This fibrin clot has a high cohesion because of the agglutination of fibrinogen, factor XIII and thrombin. Factor XIIIa, which is activated by thrombin, causes fibrin to clot. This provides protection from plasmin degradation, resulting in higher fibrin tensile strength and stability (Rodella et al. 2011, Kim et al. 2014b).

PRP and PRF have been used for repair of intra-bony defects (Camargo et al. 2002, Thorat et al. 2011), furcation defects (Lekovic et al. 2003, Sambhav et al. 2014) and sinus augmentation (Froum et al. 2002, Tajima et al. 2013) as promoters of tissue re-generation (Anilkumar et al. 2009). All these procedures have demonstrated new bone formation and bone healing. Both of them are used in the recon-

struction of soft periodontal tissue such as treatment of GRs (Aroca et al. 2009, Naik et al. 2013, Eren & Atilla 2014).

Recently, investigators reported the use of CGF in the re-construction of the bone defects. They concluded that bone formation had significantly increased by use of CGF (Kim et al. 2014b). It has been used to accelerate new bone formation associated with guided bone re-generation in sinus augmentation (Sohn et al. 2011, Choi et al. 2014). Sohn et al. (2011) stated that CGF may have a better re-generative capacity and high versatility. Therefore, this study hypothesized that CGFs' placement together with CAF may enhance the healing of soft tissues. Therefore, the aim of this study was to determine the clinical effect of CGF in combination with CAF compared to CAF alone in the treatment of adjacent multiple GRs.

Material and Methods

Study population and design

The patients of this randomized, split-mouth and controlled clinical trial study protocol were selected from individuals referred to the Department of Periodontology, at the Faculty of Dentistry, Bülent Ecevit University, for either dentin hypersensitivity or aesthetic complaints between February 2013 and April 2014. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Bülent Ecevit University, Zonguldak, Turkey in accordance with the Helsinki Declaration of 1975, as revised in 2000 (Protocol ID: 2013-24-12/02, Clinical Trial.org-NCT02385734). The patients were informed about the protocol of the study and gave their written consent to the described procedures.

The subjects were enrolled to this study based on the following inclusion criteria: (1) age > 18 years, (2) systemically and periodontally healthy, (3) non-smokers, (4) presence of ≥ 2 buccal adjacent Miller Class I or II GR with ≥ 2 mm GR depth (RD), probing depth (PD) < 3 mm and gingival thickness (GT) ≥ 1 mm on both sides of the maxillary arch, (5) width of keratinized

gingiva (KGW) ≥ 2 mm, (6) presence of identifiable cemento-enamel junction (CEJ), (7) full-mouth plaque control record (PCR) $\leq 20\%$ (O'Leary et al. 1972) and gingival index (GI) scores = 0 (Loe 1967) and (8) presence of tooth vitality and absence of caries, restorations and furcation involvement in the treated area.

The criteria for exclusion were as follows: (1) patients who had systemic problems that would contraindicate for periodontal surgery, (2) usage of medications known to interfere with healing and to cause gingival enlargement, (3) recession defects associated with demineralization, deep abrasion, (4) previous surgery in the defected area within the past 1 year, (5) pregnant or lactating females and (6) drug and alcohol abuse.

All the subjects received oral hygiene instructions and full-mouth scaling were performed 1 month before surgery. They were instructed to perform a non-traumatic brushing technique (Roll) using an ultra-soft toothbrush. In twenty patients (mean age 37.10 ± 1.03 , 20–45 years, 13 females, 7 males), one side of the jaw received CAF (control; 59 defects), the opposite site received CAF + CGF (test; 60 defects). The location and distribution of treated defects is depicted in Fig. 1.

Primary and secondary outcome variables

The primary outcome variable was the assessment of complete root coverage (CRC). The secondary outcome variables included the assessment of mean root coverage (MRC), RD, PD, recession width (RW), clinical attachment level (CAL), KGW and GT.

Clinical measurements

PCR (O'Leary et al. 1972) and GI (Loe 1967) were evaluated before surgery. Custom acrylic guides were fabricated to measure the clinical parameters. PD, CAL, RD and KGW were recorded at the mid-buccal aspect of the treated teeth. PD was measured from the gingival margin to the bottom of the sulcus. CAL was measured from the CEJ to the bottom of the sulcus. RD was measured from the CEJ to the

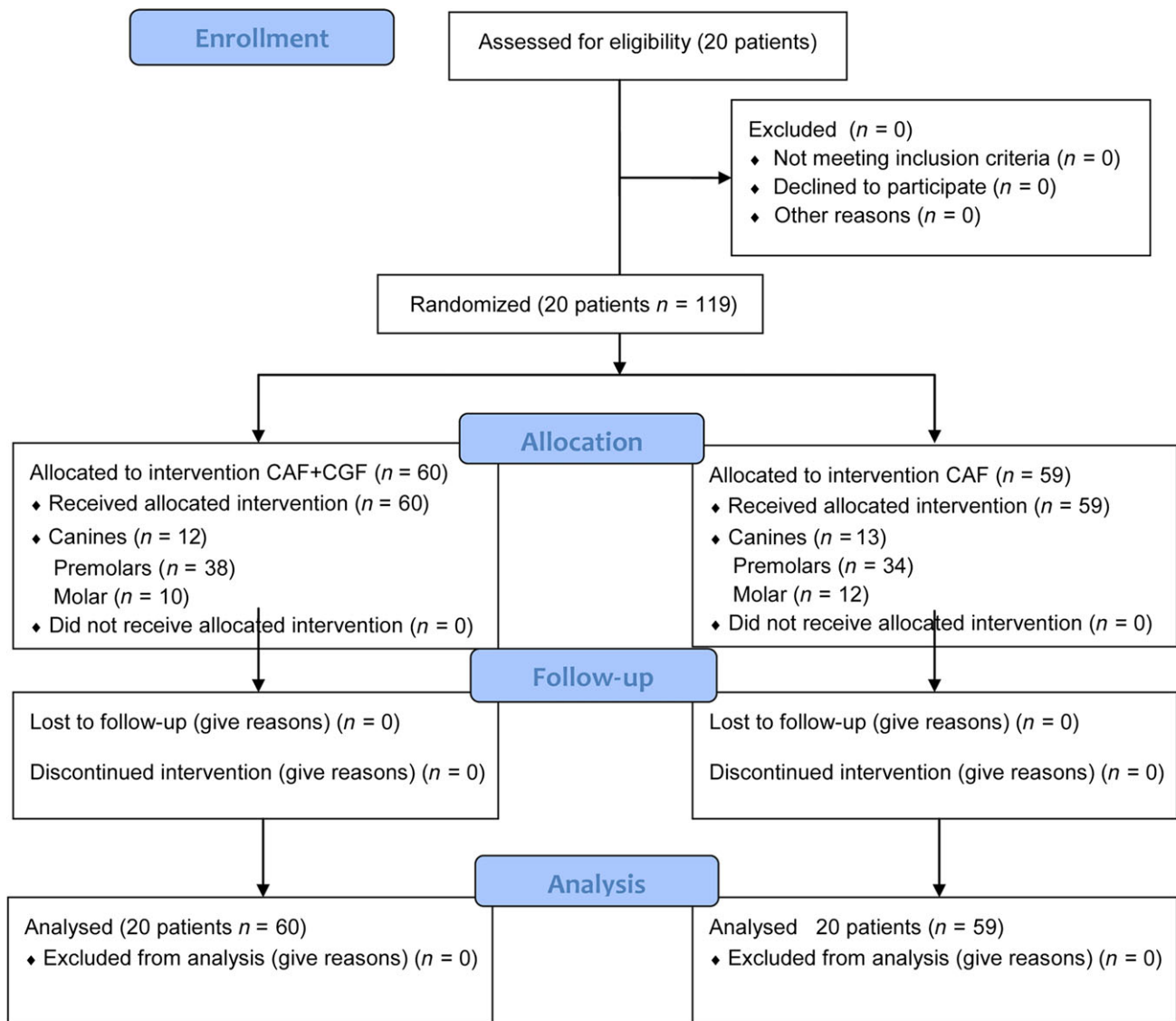


Fig. 1. Consort flowchart of the study.

gingival margin; KGW was measured from the mucogingival junction (MGJ) to the gingival margin. RW was measured from one border of the recession to another in mesio-distal direction at CEJ level. GT was evaluated mid-facially, 2 mm apical to the gingival margin at the attached gingiva or the alveolar mucosa, using a 20 endodontic reamer (Bahadır Diş Malz, İstanbul, Turkey) attached to a rubber stopper under the local anaesthesia. After carefully removing the reamer, GT was measured with a digital calliper with 0.05 resolution (Stainless Steel Digital Caliper 75 mm, Shan, China). PD, CAL, RD, RW, KGW and GT were assessed at baseline

and after 6 month. CRC and MRC were calculated in a similar way to the Naik et al.'s (2013) study.

All clinical measurements were recorded by a calibrated, single masked examiner (FÖD) using a periodontal Goldman/Fox Williams probe and rounded up to the nearest 0.5 mm (Nordent Manufacturing Inc., Elk Grove Village, IL, USA). The examiner did not perform the surgeries and was unaware of the treatment assignment. The calibration was achieved by examination of twenty defects in five patients two times in a period of 72 h. Calibration was accepted, if measurements of recession (PD, CAL, RD, KGW and GT) at baseline and at 72 h

were similar to the 0.5 mm at the 90% level (Aroca et al. 2009).

CGF Preparation

Intravenous blood was collected in two 10-ml glass-coated plastic tubes without anticoagulant solutions. These tubes were then immediately centrifuged with a CGF centrifuge machine (Medifuge, Silfradentsr, S. Sofia, Italy) using a program with the following characteristics: 30" acceleration, 2' 2700 r.p.m., 4' 2400 r.p.m., 4' 2700 r.p.m., 3' 3000 r.p.m., and 36" deceleration and stop. At the end of the centrifugation there were four blood fractions: (1) the upper serum layer, (2) the second buffy coat

layer, (3) the third GF and unipotent stem cell layer (CGF) and (4) the lower red blood cell layer (RBC). The CGF clot was removed from the tube and separated from the RBC by using microsurgical scissors. The CGF was squeezed in a special box that produces membranes at a constant thickness of 1 mm (Fig. 2a, b). The CGF membrane was immediately placed over the recession area.

Surgical procedure

All surgeries were performed by the same expert periodontist (ŞBD) during a single surgical session. GR sites were randomly determined as either test or control site by tossing a coin immediately before the surgical procedure.

After local anaesthesia using 2% lidocaine, epinephrine at 1:1,000,000, sulcular incisions were made on the teeth and joined to horizontal incisions extending into the adjacent inter-dental areas slightly coronal to the CEJ. Two vertical incisions were made extending beyond the MGJ at mesio-facial and disto-facial line angles of the study teeth. A trapezoidal mucoperiosteal flap was elevated by blunt dissection to the level of MGJ. A partial thickness flap was initiated at the MGJ and a sharp dissection was carried in the apical direction to the point at which the flap could be coronally positioned and would sit passively, without any tension at the level of CEJ. All incisions were made using a 15-C blade (Swann-Morton LTD, Sheffield, UK). All papillae were deepithelialized to create a connective tissue bed.

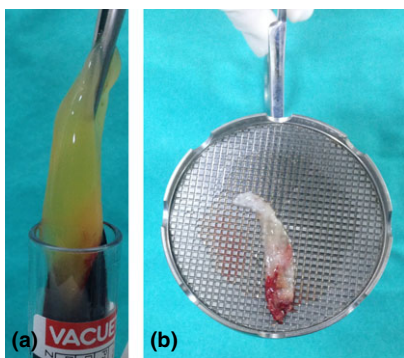


Fig. 2. (a) Concentrated growth factor (CGF) clot after centrifugation and (b) squeezed CGF membrane in a special box.

The exposed root surfaces were planed using curettes (Gracey curettes; Hu Friedy, Chicago, IL, USA) to remove plaque, calculus and soft tooth structure. No further root conditioning, mechanical or chemical, was performed. These procedures were the same in both of the groups. The CGF membrane was placed over the defect and extended apically beyond the apical base of recession defects by ≥ 3 mm in the test group (Fig. 3b). The flap was coronally positioned over the membrane to completely cover the CEJ and sutured with 4-0 poly (glycolide-co-lactide) absorbable sutures (Doğsan sutures; Trabzon, Turkey) in the test group (Fig. 3c). The same procedure was performed without CGF membrane in the control group. Stabilization of blood clot was obtained with a gentle pressure for 3 min (Aroca et al. 2009, Eren & Atilla 2014).

Post-operative care

About 550 mg naproxen sodium (Apranax Forte 550 mg; Abdi Ibrahim, Turkey) was given for post-operative pain and oedema every 8 h as needed. Patients were informed not to eat solid food using the treated area and not to brush their teeth in the treated area, but to rinse with chlorhexidine digluconate (0.2%, CHX) two times a day for 1 min.

The sutures were removed after 10 days and the plaque control was maintained by CHX for additional 2 weeks. The patients started brushing their tooth at the end of the third week and they were again instructed in mechanical tooth cleaning of the treated tooth using an ultra-soft toothbrush and roll technique. Oral hygiene instructions were provided at each post-operative visit.

Statistical analysis

Prior to the initiation of the study, the power analysis for sample size calculation was performed. According to the results of power analysis, 14 patients were needed for each group to have 80% power to detect a minimum clinically significant difference in RC of 1 mm with a standard deviation of 0.9 mm (McGuire & Scheyer 2010, Eren & Atilla 2014). To allow for possible dropouts, 20 patients were finally recruited.

The mean of the respective variable over all recessions in the respective treatment group of the respective patient were used for the analysis. The data were tested for normality using the Shapiro-Wilk test. Wilcoxon signed-rank test (paired observations) was carried out to compare PD, CAL, RD, RW, KGW, GT and MRC between the groups after normality of data failed.

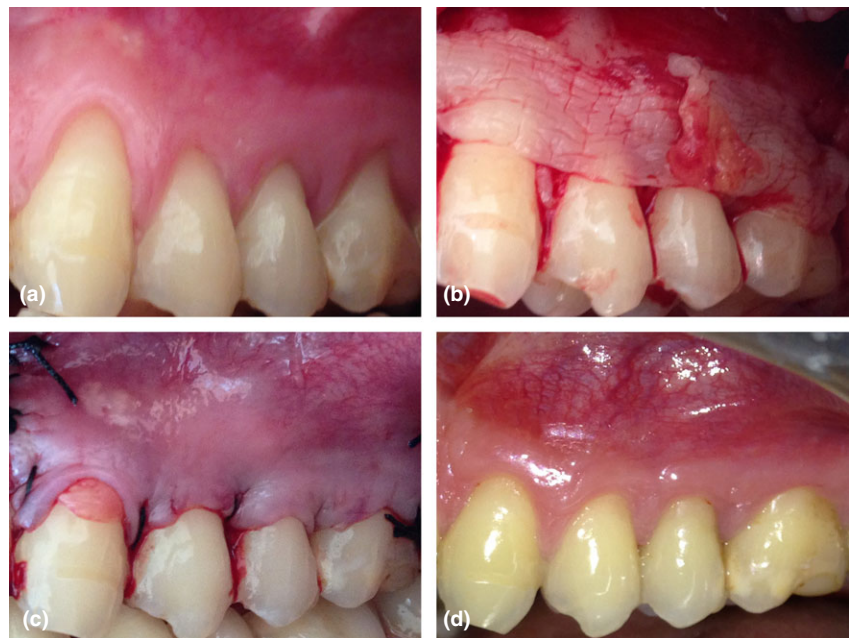


Fig. 3. Test group: (a) pre-operative view, (b) intra-operative view, (c) immediate post-operative view, and (d) 6 months post-operative view.

Wilcoxon signed-rank test (paired observations) was used to compare the baseline values with those after 6 months. Chi-square analysis was used to compare test and control groups concerning CRC at 6 months. All tests were performed using statistical software (version 15.0; SPSS Inc., Chicago, IL, USA). The mean \pm standard deviation with mean rank values were calculated for the parameters on the basis that the patients are the statistical unit. $p < 0.05$ was considered to be statistically significant.

Results

All 20 patients completed the study. None patient was excluded from the study (Fig. 1). All the surgical sites healed uneventfully. No adverse events related to both treatment modalities were recorded. An analysis of the defect characteristics revealed no significant differences between two groups for any of the considered clinical parameters at baseline. The patients demonstrated good plaque control with $\leq 20\%$ PCR and equal to 0 GI scores at the baseline and 6 months evaluation. Comparisons between the baseline and after 6 month clinical view of patients are shown in Figs 3a,d and 4.

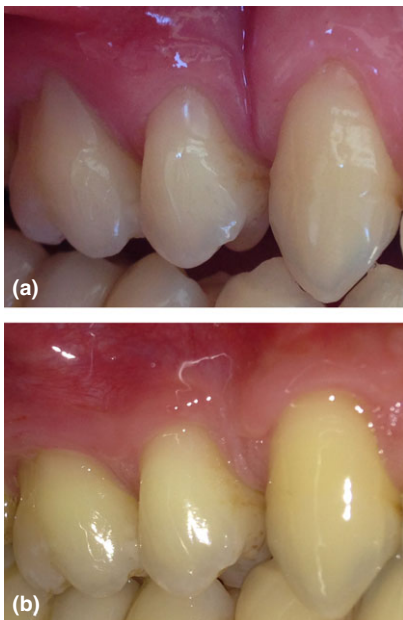


Fig. 4. Control group: (a) pre-operative view and (b) 6 months post-operative view.

Clinical outcome

RD and CRC

Both the treatment groups showed a statistically significant reduction in RD at 6 months compared to baseline ($p < 0.05$; Table 1). RD decreased by 2.29 ± 0.56 , which represents MRC 82.06% in the control group. RD decreased by 2.47 ± 0.54 , which represents MRC 86.67% in the test group. CRC was found in 45.8% of the sites (27/59) in the control group and in 56.7% of the sites (34/60) in the test group (Table 2). There was no statistically significant difference in RD, RC and CRC between the two groups ($p > 0.05$). MRC was shown to be 86.56 ± 15.29 in the control group and 91.15 ± 14.11 in the test group if the first molars are excluded and the difference was not significant ($p > 0.05$).

PD

A statistically significant decrease was observed in both the groups at 6 months compared to baseline ($p < 0.05$). In the inter-group comparison at 6 months, there was no statistically significant difference between control and test group ($p > 0.05$; Table 1).

CAL

A Significant gain was observed in both the control and test group at 6 months compared to the baseline measurements ($p < 0.05$). A statistically greater gain was observed in the test group ($p < 0.05$). In the inter-group comparison at 6 months, there was no statistically significant difference between control and test group ($p > 0.05$; Table 1).

RW

Both the treatment groups showed a statistically significant reduction in RW at 6 months compared to baseline ($p < 0.05$). There was no statistically significant difference in the inter-group comparison at 6 months ($p > 0.05$; Table 1).

KGW

A Significant increase was observed in the test group ($p < 0.05$), while no difference was found in the control group at 6 months compared to the baseline ($p > 0.05$). At 6 months, the amount of KGW was significantly

higher in the test group compared to the control group ($p < 0.05$; Table 1).

GT

A Significant increase was observed in both the control and test group at 6 months compared to the baseline measurements. A statistically greater increase was observed in the test group compared to the control group ($p < 0.05$). In the inter-group comparison at 6 months, GT in the test group was significantly higher compared to the control group ($p < 0.05$; Table 1).

Discussion

GFs are bioactive proteins that control the process of wound healing. GFs have a critical role in cell migration, cell proliferation and angiogenesis for tissue re-generation (Clark 2001). One of the possible ways to improve the clinical results of GR treatment is to use GFs (Lafzi et al. 2012). Most of the studies in this field have investigated the effects of PRP and PRF on RC procedure. (Huang et al. 2005, Keceli et al. 2008, Aroca et al. 2009, Eren & Atilla 2014). The effects of autogenous PCs on clinical outcomes of the surgical treatment of periodontal diseases were evaluated in a systematic review (Del Fabbro et al. 2011). They concluded that PCs did not have significant benefit for the treatment of GR. However, in another systematic review, Luo et al. (2015) concluded that the additional use of PCs might exert a positive effect in the treatment of GR and wound healing. Since then, there were an increasing number of researches about the application of PCs in the treatment of GR. Recently, the use of CGF, as an alternative PC, has been reported with limited data (Sohn et al. 2009, 2011, Kim et al. 2014a). There is no published data about the use of CGF in periodontal plastic surgery. To the best of our knowledge, this study is the first clinical trial that evaluated the clinical effects of using CGF membrane in the treatment of adjacent multiple GRs.

CRC ensure recovery from hypersensitivity and aesthetic factors associated with recession. Huang et al. examined the effects of PRP in combination with CAF in single GRs. They found that RD was reduced to

Table 1. Descriptive statistics of the clinical parameters measured at baseline and 6 months after surgery

	Control group (n = 59)	Test group (n = 60)	p-Value
PD			
Baseline	1.66 ± 0.48 (58.33)	1.72 ± 0.45 (61.64)	0.564
6 months	1.37 ± 0.49 (60.69)	1.35 ± 0.48 (59.33)	0.847
Difference	0.29 ± 0.46	0.37 ± 0.49	0.414
p-Value	0.000	0.000	
CAL			
Baseline	4.51 ± 0.65 (57.01)	4.62 ± 0.64 (62.94)	0.499
6 months	1.93 ± 0.41 (64.24)	1.78 ± 0.52 (55.83)	0.083
Difference	2.58 ± 0.62	2.83 ± 0.62	0.043
p-Value	0.000	0.000	
RD			
Baseline	2.85 ± 0.69 (58.60)	2.90 ± 0.63 (61.38)	0.771
6 months	0.56 ± 0.53 (63.49)	0.43 ± 0.50 (56.57)	0.144
Difference	2.29 ± 0.56	2.47 ± 0.54	0.138
p-Value	0.000	0.000	
RW			
Baseline	3.78 ± 1.55 (59.02)	3.90 ± 1.57 (60.69)	0.737
6 months	0.86 ± 0.94 (62.68)	0.75 ± 1.00 (57.37)	0.472
Difference	2.92 ± 1.02	3.15 ± 0.88	0.208
p-Value	0.000	0.000	
KGW			
Baseline	2.49 ± 0.50 (61.00)	2.47 ± 0.54 (59.02)	0.715
6 months	2.63 ± 0.55 (50.25)	3.05 ± 0.65 (69.59)	0.001
Difference	0.14 ± 0.63	0.58 ± 0.53	0.000
p-Value	0.102	0.000	
GT			
Baseline	1.10 ± 0.07 (62.11)	1.09 ± 0.07 (57.93)	0.568
6 months	1.16 ± 0.10 (32.58)	1.40 ± 0.10 (86.97)	0.000
Difference	0.06 ± 0.09	0.32 ± 0.10	0.000
p-Value	0.000	0.000	

Data are expressed as the mean ± standard deviation (Mean Rank).

CAL, Clinical attachment level; GT, Gingival thickness; KGW, Keratinized gingiva width; PD, Probing pocket depth; RD, Gingival recession depth.

Values in bold are statistically significant ($p < 0.05$), Wilcoxon signed-rank test.

Table 2. Mean and SD of the root coverage percentage and complete root coverage in the operated patients at 6 months post-operatively

	Control group (n = 59)	Test group (n = 60)	p-Value
Mean root coverage	82.06 ± 17.49	86.67 ± 15.59	0.170*
Complete root coverage	27/59 (45.8%)	34/60 (56.7%)	0.234†

Data are expressed as the mean ± standard deviation.

*Wilcoxon signed-rank test.

†Chi-square test.

2.4 mm in CAF and 2.3 mm in CAF + PRP. CRC were obtained 58.3% and 63.6% for the CAF and CAF + PRP groups, respectively (Huang et al. 2005). Similarly, results of this study showed that both treatment groups have significant decrease in RD, and CRC was obtained in 45.8% of the control and 56.7% of the test group. However, Aroca et al. investigated that clinical effect of PRF combined with modified CAF (MCAF) in multiple GRs. They obtained CRC at 74.6%

of the sites treated with MCAF alone and 52.2% of the MCAF + PRF (Aroca et al. 2009). Our study design was similar to Aroca's study. But this study found no differences in RD and CRC between groups.

Huang et al. (2005) reported in their controlled study that the CAF + PRP combination did not present any different results compared with CAF alone in the RC. Eren & Atilla (2014) reported that RC was 94.2% for the CTG + CAF

and 92.7% CAF + PRF groups and no difference was found between the groups. Our results showed no statistically significant difference for the MRC between the groups. On the contrary to these studies, Padma et al. (2013) concluded that MRC was 100% in CAF + PRF group and differences between the CAF alone and CAF + PRF groups was statistically significant. Aroca et al. (2009) found that MRC was $80.7 \pm 14.7\%$ in the MCAF + PRF and $91.5 \pm 11.4\%$ in the MCAF alone and the difference between the groups was statistically significant. We should state that our study design was different from the above studies because CGF was used in the treatment of GRs. The biological properties among the CGF, PRP and PRF may be different from each other. These various results might be caused by the use of different surgical procedures. Since the depth of the recession defects and MCAF design and use of PRF in Aroca et al.'s study were different from this study, it could lead to differences in outcomes.

The presence of keratinized gingiva is an important factor for the maintenance of gingival health and prevention of periodontal disease progression (Lang & Loe 1972, Friedman et al. 1992). Silva et al. (2007) and Cheung & Griffin (2004) found an increase in KGW for the CAF alone and CAF-PC grafts, respectively. Padma et al. (2013) found a statistically significant increase in KGW in the CAF + PRF groups. Aroca et al. showed a significant increase in GT in the MCAF + PRF group. However, they showed a significant decrease in KGW for both of the MCAF + PRF and MCAF alone groups (Aroca et al. 2009). In this study, both groups showed an increase in KGW and GT, the increase in KGW and GT was significantly higher in the test group compared to the control group after 6 months. In the Pini Prato's study, an apical relapse of the gingival margin was observed in CAF alone-treated sites between the 6 month and 5 year follow-ups (Pini-Prato et al. 2010). This negative trend following CAF was attributed to less thickness/amount of keratinized tissue achieved. The clinical increase in GT and KGW

represent the stability of root coverage in the long-term (Zucchelli et al. 2014). In this study, the higher increase in KGW and GT in the test group may be explained by biology of CGF, which contains much larger, denser and richer in GFs fibrin matrix (Sohn et al. 2009, Rodella et al. 2011). But, this statement must be confirmed with further histologic studies.

In this study, our data showed a significant decrease of PD in both of the groups. On the other hand, the difference between the two groups after 6 month was not statistically significant, which is in agreement with Aroca et al.'s (2009) study. Additionally, in our study, a significant CAL gain and a decrease in RW were observed in both of groups at 6 months, which is compatible with other studies (Huang et al. 2005, Aroca et al. 2009, Padma et al. 2013).

This study was designed as randomized, split-mouth and controlled clinical trial in multiple bilateral adjacent teeth. Multiple adjacent recessions are more challenging defects, as the surgical field is larger and more anatomic variations may be present (Aroca et al. 2009). It has to be pointed out that this study included multiple bilateral GRs located at maxillary molars, which may increase surgical difficulties and risk of failure compared with monolateral and single-tooth GRs. Additionally, RW can be wider in molars than anterior teeth. Due to a wider RW, a lesser RC should be expected (Shieh et al. 1997). The overall results of the study was, most likely, influenced by the inclusion of molars. If molar GRs were excluded from the study groups, MRC was 86.56 ± 15.29 in the control group, and 91.15 ± 14.11 in the test group. The reason of including molars was the fact that these posterior sites may be of concern for patients exhibiting root hypersensitivity and compromised aesthetics. A 6-months post-operative measurement period is sufficient to evaluate the stability of the gingival margin after a CAF (Cheng et al. 2007). Therefore, we evaluated the post-operative measurements after 6 month, but additional healing might occur over longer periods (Kimble et al. 2004).

In this study, histological examination was not performed to determine the re-generative capacity of CGF. These limitations may have affected the final clinical results. Further studies are needed to evaluate these issues.

Conclusion

Within the limitation of this study, it can be concluded that CGF + CAF was not superior to CAF alone in providing a consistent reduction in the baseline recession. If the therapy's objectives are to increase GT and KGW, the use of CGF combined with CAF should be considered. This KGW and GT augmentation might improve the long-term predictability of this procedure, by diminishing post-surgical relapse and thus providing long-term stability.

References

- Anilkumar, K., Geetha, A., Umasudhakar, T. R., Vijayalakshmi, R. & Pameela, E. (2009) Platelet-rich-fibrin: a novel root coverage approach. *Journal of Indian Society of Periodontology* **13**, 50–54.
- Aroca, S., Keglevich, T., Barbieri, B., Gera, I. & Etienne, D. (2009) Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study. *Journal of Periodontology* **80**, 244–252.
- Camargo, P. M., Lekovic, V., Weinlaender, M., Vasilic, N., Madzarevic, M. & Kenney, E. B. (2002) Platelet-rich plasma and bovine porous bone mineral combined with guided tissue regeneration in the treatment of intrabony defects in humans. *Journal of Periodontal Research* **37**, 300–306.
- Chambrone, L. & Tatakis, D. N. (2015) Periodontal soft tissue root coverage procedures: a systematic review from the AAP Regeneration Workshop. *Journal of Periodontology* **86**, S8–S51.
- Cheng, Y. F., Chen, J. W., Lin, S. J. & Lu, H. K. (2007) Is coronally positioned flap procedure adjunct with enamel matrix derivative or root conditioning a relevant predictor for achieving root coverage? A systemic review. *Journal of Periodontal Research* **42**, 474–485.
- Cheung, W. S. & Griffin, T. J. (2004) A comparative study of root coverage with connective tissue and platelet concentrate grafts: 8-month results. *Journal of Periodontology* **75**, 1678–1687.
- Choi, Y. S., Kim, Y. C., Ji, S. & Choi, Y. (2014) Increased bacterial invasion and differential expression of tight-junction proteins, growth factors, and growth factor receptors in periodontal lesions. *Journal of Periodontology* **85**, e313–e322.
- Clark, R. A. (2001) Fibrin and wound healing. *Annals of the New York Academy of Sciences* **936**, 355–367.
- Del Fabbro, M., Bortolin, M., Taschieri, S. & Weinstein, R. (2011) Is platelet concentrate advantageous for the surgical treatment of periodontal diseases? A systematic review and meta-analysis. *Journal of Periodontology* **82**, 1100–1111.
- Eren, G. & Atilla, G. (2014) Platelet-rich fibrin in the treatment of localized gingival recessions: a split-mouth randomized clinical trial. *Clinical Oral Investigation* **18**, 1941–1948.
- Friedman, M. T., Barber, P. M., Mordan, N. J. & Newman, H. N. (1992) The "plaque-free zone" in health and disease: a scanning electron microscope study. *Journal of Periodontology* **63**, 890–896.
- Froum, S. J., Wallace, S. S., Tarnow, D. P. & Cho, S. C. (2002) Effect of platelet-rich plasma on bone growth and osseointegration in human maxillary sinus grafts: three bilateral case reports. *International Journal of Periodontics Restorative Dentistry* **22**, 45–53.
- Graziani, F., Gennai, S., Roldan, S., Discepoli, N., Buti, J., Madianos, P. & Herrera, D. (2014) Efficacy of periodontal plastic procedures in the treatment of multiple gingival recessions. *Journal of Clinical Periodontology* **41** (Suppl 15), S63–S76.
- Huang, L. H., Neiva, R. E., Soehren, S. E., Gianobile, W. V. & Wang, H. L. (2005) The effect of platelet-rich plasma on the coronally advanced flap root coverage procedure: a pilot human trial. *Journal of Periodontology* **76**, 1768–1777.
- Keceli, H. G., Sengun, D., Berberoglu, A. & Karabulut, E. (2008) Use of platelet gel with connective tissue grafts for root coverage: a randomized-controlled trial. *Journal of Clinical Periodontology* **35**, 255–262.
- Kim, J. M., Sohn, D. S., Bae, M. S., Moon, J. W., Lee, J. H. & Park, I. S. (2014a) Flapless transcresal sinus augmentation using hydrodynamic piezoelectric internal sinus elevation with autologous concentrated growth factors alone. *Implant Dentistry* **23**, 168–174.
- Kim, T. H., Kim, S. H., Sandor, G. K. & Kim, Y. D. (2014b) Comparison of platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and concentrated growth factor (CGF) in rabbit-skull defect healing. *Archives of Oral Biology* **59**, 550–558.
- Kimble, K. M., Eber, R. M., Soehren, S., Shyr, Y. & Wang, H. L. (2004) Treatment of gingival recession using a collagen membrane with or without the use of demineralized freeze-dried bone allograft for space maintenance. *Journal of Periodontology* **75**, 210–220.
- Lafzi, A., Faramarzi, M., Shirmohammadi, A., Behroozian, A., Kashefimehr, A. & Khashabi, E. (2012) Subepithelial connective tissue graft with and without the use of plasma rich in growth factors for treating root exposure. *Journal of Periodontal Implant Science* **42**, 196–203.
- Lang, N. P. & Loe, H. (1972) The relationship between the width of keratinized gingiva and gingival health. *Journal of Periodontology* **43**, 623–627.
- Lekovic, V., Camargo, P. M., Weinlaender, M., Vasilic, N., Aleksic, Z. & Kenney, E. B. (2003) Effectiveness of a combination of platelet-rich plasma, bovine porous bone mineral and guided tissue regeneration in the treatment of mandibular grade II molar furcations in humans. *Journal of Clinical Periodontology* **30**, 746–751.
- Loe, H. (1967) The gingival index, the plaque index and the retention index systems. *Journal of Periodontology* **38** (Suppl), 610–616.

- Luo, H. Y., Li, R. M., Wang, C. L., Peng, L. & Ye, L. (2015) The adjunctive use of platelet concentrates in the therapy of gingival recessions: a systematic review and meta-analysis. *Journal of Oral Rehabilitation* **42**, 552–561.
- McGuire, M. K. & Scheyer, E. T. (2010) Xenogeneic collagen matrix with coronally advanced flap compared to connective tissue with coronally advanced flap for the treatment of dehiscence-type recession defects. *Journal of Periodontology* **81**, 1108–1117.
- Naik, A. R., Ramesh, A. V., Dwarkanath, C. D., Naik, M. S. & Chinnappa, A. B. (2013) Use of autologous platelet rich plasma to treat gingival recession in esthetic periodontal surgery. *Journal of Indian Society of Periodontology* **17**, 345–353.
- Nieri, M., Pini Prato, G. P., Giani, M., Magnani, N., Pagliaro, U. & Rotundo, R. (2013) Patient perceptions of buccal gingival recessions and requests for treatment. *Journal of Clinical Periodontology* **40**, 707–712.
- O'Leary, T. J., Drake, R. B. & Naylor, J. E. (1972) The plaque control record. *Journal of Periodontology* **43**, 38.
- Padma, R., Shilpa, A., Kumar, P. A., Nagasri, M., Kumar, C. & Sreedhar, A. (2013) A split mouth randomized controlled study to evaluate the adjunctive effect of platelet-rich fibrin to coronally advanced flap in Miller's class-I and II recession defects. *Journal of Indian Society of Periodontology* **17**, 631–636.
- Pini-Prato, G. P., Cairo, F., Nieri, M., Franceschi, D., Rotundo, R. & Cortellini, P. (2010) Coronally advanced flap versus connective tissue graft in the treatment of multiple gingival recessions: a split-mouth study with a 5-year follow-up. *Journal of Clinical Periodontology* **37**, 644–650.
- Rodella, L. F., Favero, G., Boninsegna, R., Bufoli, B., Labanca, M., Scari, G., Sacco, L., Batani, T. & Rezzani, R. (2011) Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. *Microscopy Research Technique* **74**, 772–777.
- Sambhav, J., Rohit, R., Ranjana, M. & Shalabh, M. (2014) Platelet rich fibrin (PRF) and betacalcium phosphate with coronally advanced flap for the management of grade-II furcation defect. *Ethiopian Journal of Health Sciences* **24**, 269–272.
- Shieh, A. T., Wang, H. L., O'Neal, R., Glickman, G. N. & MacNeil, R. L. (1997) Development and clinical evaluation of a root coverage procedure using a collagen barrier membrane. *Journal of Periodontology* **68**, 770–778.
- Silva, C. O., de Lima, A. F., Sallum, A. W. & Tatakis, D. N. (2007) Coronally positioned flap for root coverage in smokers and non-smokers: stability of outcomes between 6 months and 2 years. *Journal of Periodontology* **78**, 1702–1707.
- Sohn, D. S., Heo, J. U., Kwak, D. H., Kim, D. E., Kim, J. M., Moon, J. W., Lee, J. H. & Park, I. S. (2011) Bone regeneration in the maxillary sinus using an autologous fibrin-rich block with concentrated growth factors alone. *Implant Dentistry* **20**, 389–395.
- Sohn, D. S., Moon, J. W., Moon, Y. S., Park, J. S. & Jung, H. S. (2009) The use of concentrated growth factors (CGF) for sinus augmentation. *Journal of Oral Implant Science* **38**, 25–38.
- Tajima, N., Ohba, S., Sawase, T. & Asahina, I. (2013) Evaluation of sinus floor augmentation with simultaneous implant placement using platelet-rich fibrin as sole grafting material. *International Journal of Oral Maxillofacial Implants* **28**, 77–83.
- Thorat, M., Pradeep, A. R. & Pallavi, B. (2011) Clinical effect of autologous platelet-rich fibrin in the treatment of intra-bony defects: a controlled clinical trial. *Journal of Clinical Periodontology* **38**, 925–932.
- Zucchelli, G., Marzadori, M., Mounssif, I., Mazzotti, C. & Stefanini, M. (2014) Coronally advanced flap + connective tissue graft techniques for the treatment of deep gingival recession in the lower incisors. A controlled randomized clinical trial. *Journal of Clinical Periodontology* **41**, 806–813.

Address:
 Şeyma Bozkurt Doğan
 Department of Periodontology
 Faculty of Dentistry
 Bülent Ecevit University
 Tıp Fakültesi Caddesi
 67100, Zonguldak
 Turkey
 E-mail: dtseyma@hotmail.com

Clinical Relevance

Scientific rationale for the study: The use of adjunctive agents of platelet concentrates is one of the therapies to accelerate wound healing and repair. There is no study about the evaluation of clinical effect of CGF in the treatment of GRs.

Principal findings: Additional benefits of CGF in clinical outcomes were not found between the two groups except on the amount of KGW and GT. Both of the groups showed statistically significant MRC.

Practical implications: The use of CAF + CGF should be suggested

because of the significant increase in KGW and GT for the treatment of multiple GR. More randomized clinical trial are needed to test whether the CGF have an adjunctive effect on root coverage procedures.