Original Article

Efficiency of platelet-rich plasma on acellular dermal matrix application with coronally advanced flap in the treatment of multiple adjacent gingival recessions: A randomized controlled clinical trial

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Abstract *Background/Purpose:* The aim of this study was to evaluate the effectiveness of platelet rich plasma (PRP) combined with coronally advanced flap plus acellular dermal matrix application (CAF + ADM) in the treatment of multiple adjacent gingival recessions (MAGRs).
Materials and methods: 12 patients with 84 Miller Class I or II recession defects were participated. Sites were randomly assigned into CAF + ADM + PRP or CAF + ADM groups. Gingival recession depth (GRD), recession width (GRW), width of keratinized tissue (WKT), creeping attachment (CRA), root coverage (RC) as well as plaque index (PI), gingival index (GI), probing depth (PD), and clinical attachment level (CAL) were recorded at baseline and 3rd,6th and 12th months postoperatively. The data were analyzed statistically.
Results: GRD and GRW values were statistically higher in group CAF + ADM compared to the CAF + ADM + PRP group at 6th and 12th month ($P < 0.05$). CRA showed statistically significant increases in 3rd, 6th and 12th months with regard to the baseline in CAF + ADM + PRP group ($P < 0.05$), however CRA remained stable in CAF + ADM group at 6th and 12th month. The mean RC% was 77.9% and 69.4% for the CAF + ADM + PRP and CAF + ADM groups, respectively, at 12th month ($P < 0.05$). GRD reduction was statistically greater in the maxillary teeth treated with CAF + ADM + PRP compared to mandibular teeth treated with CAF + ADM at 12 months.

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Conclusion: Our data suggested that addition of PRP to CAF + ADM in the treatment of MAGRs significantly improved the clinical outcomes, according to 1-year follow-up results.
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Treatment of multiple adjacent gingival recessions (MAGRs) with the goal of complete root coverage, poses specific challenges for the clinician.

Various periodontal plastic surgical techniques have been proposed for the treatment of MAGRs including coronally advanced flaps (CAF) with or without a connective tissue graft (CTG), modified CAFs, and modified coronally advanced tunnel technique. Among all, CAF together with CTG is regarded as the gold standard for treatment of gingival recessions. However, even CAF + CTG technique has its limitations, especially for patients with MAGRs, such as morbidity and limited quantity of donor tissue. Moreover, wound healing may be compromised in MAGRs due to the differences in recession depths and tooth position due to the width of the avascular surface.[1–3]

Acellular dermal matrix (ADM) is a lyophilized or freeze-dried human skin allograft, in which the cell component, the target of rejection response, is removed.[4] It has a basement membrane and a connective tissue side including collagen and laminin. ADM works best when completely covered by host tissue, resulting in a perfect color match[5] and predictable host tissue thickness.[6] A CAF underlaid by ADM suggested to achieve similar outcomes with CAF + CTG in terms of defect coverage percentage and predictability.[7] ADM also reported to increase the zone of attached gingiva around teeth and mucosa around dental implants.[8,9]

Lately, platelet rich plasma (PRP), an autologous platelet concentration, suggested to have a potential to increase regeneration and accelerate wound healing,[11] due to the various growth factors it consists.[10–13] PRP contains a plethora of substances involved in the wound healing process that have the potential to greatly enhance soft tissue healing.[14] Increased vascularity, wound stability, esthetics, regeneration potential and decreased patient morbidity are suggested as the advantages of PRP.[14]

Although there are numerous root coverage procedures to treat the conditions, predictable coverage of MAGRs still remains a challenge for the clinician. The aim of this randomized, controlled, clinical trial was to compare the CAF plus ADM application together with and without PRP to determine whether the PRP provided an advantage in terms of gaining coverage of deep and wide recession defects in the treatment of MAGRs.

Materials and methods

Study design and subject selection

In the present study, using a controlled blinded split-mouth design, 14 patients with multiple Miller Class I or II adjacent recession defects ≥ 3 mm deep on non-molar teeth in the same dental arch at least two bilateral sites, were randomly operated either with CAF + ADM + PRP (Sure-Derm, Seoul, Korea) or only CAF + ADM as test and control groups, respectively. Two patients who failed to return for all postoperative visits were exited from the study. The study sample consisted of 84 teeth associated with total of 12 patients; seven patients each showing a pair of three teeth, four patients each showing a pair of four teeth, and one patient showing a pair of five teeth with adjacent multiple recessions deep on the buccal aspect of each tooth. The treated teeth were 26 incisors, 24 canines and 34 first premolars (Table 1) and were followed for 12 months. Standardized radiographs were taken to evaluate the interproximal alveolar bone level.

Criteria for subject selection were as follows: (1) the presence of at least three multiple adjacent Miller class I or II recessions on both sides of the same maxillary or mandibular arch (2) presence of ≤2 mm-high keratinized tissue apical to the root exposures; (3) no systemic diseases that could influence the outcome of the therapy; (4) a full-mouth plaque score of 20%; (5) non-smoker; (6) not pregnant.

Patients were excluded if any of the following criteria were present: debilitating systemic or infectious diseases (human immunodeficiency virus or hepatitis) or any disease that significantly affects the periodontium; a known allergy to any of the materials used in the study; requirement for antibiotic prophylaxis; taking medications known to interfere with periodontal health and healing not detectable cemento-enamel junction (CEJ); restorations or caries at the recession site; failure to maintain an oral hygiene level ≥80% plaque-free surfaces; pregnancy or lactation; use of tobacco products; alcohol abuse; a previous periodontal

Table 1 Distributions of operated teeth according to groups.

Location	Study Groups		
	CAF + ADM + PRP	CAF + ADM	TOTAL
Maxillary			
incisor	6	6	12
canine	6	6	12
premolar	7	7	14
Mandibular			
incisor	7	7	14
canine	6	6	12
premolar	10	10	20
TOTAL	**42**	**42**	**84**

surgery at the recession site and failure to complete the informed consent.

In each patient, one side of the mandible (or maxilla) served as positive control and the opposite side as test. The same experienced practitioner (P.G) performed both operations (at test and control sites) during a single surgical session.

The protocol of this clinical trial was approved by the Ethical Committee of the Faculty of Dentistry, Gazi University. Participants of this study were recruited from individuals referred to the Department of Periodontology, Faculty of Dentistry of Gazi University. The study was performed in accordance with the Helsinki Declaration of 1975, as revised in Tokyo 2004. Informed written consent was obtained from all subjects after the details of the clinical procedures, including periodontal measurements and periodontal treatment were fully explained.

Periodontal examination

At baseline, and 3, 6, 12 months after the surgeries, the following clinical parameters were recorded by one blinded investigator (ZTÇ), using a Williams periodontal probe (Nordent Manufacturing Inc., Elk Grove Vilage, IL). The examiner did not perform the surgeries and was calibrated prior to the study to reduce intra-examiner variability (kappa index >0.90).

The clinical parameters evaluated were as follows: Miller classification of the gingival recession defect,[16] PI[17], GI[18], PD, CAL, GRD, GRW, WKT, CRA and RC. PD, CAL and GRD were measured at the same three reference points (mesio-buccal, midbuccal, and disto-buccal) on the individual elastomeric stent. The GRD was measured from the CEJ to the gingival margin (GM). GRW was measured with perpendicular positioning of the periodontal probe to the CEJ, the distance between the top of the papilla at the mesial and distal aspect of the tooth was recorded. WKT was measured at the mid-buccal point from the mucogingival junction to the free GM by a digital caliper. CRA gain measured from post-surgical 8 weeks until the final examination. The percentage of RC was also calculated as ([GRD preoperation − GRD postoperation]/GRD preoperation) x 100%.

Pre-treatment procedures

The presurgical evaluation included an analysis of the patient's tooth brushing technique and habits. At the teeth showing gingival recessions, a coronally directed roll technique using a soft toothbrush was indicated to minimize the tooth brushing trauma to the gingival margin.[19] Presurgical therapy included scaling, root planing, polishing and general oral hygiene instruction. Pre- and postoperative standardized radiographs were taken to evaluate the interproximal alveolar bone level. The surgical procedure was not scheduled until the recession defects did not display either plaque deposits or bleeding on probing.[20]

PRP preparation

A sterile disposable monovette system (Curasan, Pharma Gmbh AG, Lindigstrab, Germany) and compatible centrifuge machine (Heraeus Labofuge 300, Kendro Laboratory Products, D-37520 Osterrade, German) were used for preparations of PRP as described before.[21] Briefly, eight ml of peripheral blood was drawn from each patient by venipuncture into a 0.5 ml citrate (10% trisodium citrate) containing monovettes and centrifuged at 2400 rpm for 10 min. A total of 4 mm of plasma, which consisted of the complete upper yellow layer and the lower red layer's top 1−2 mm part, was transferred into another monovette. After the second centrifugation at 3600 rpm for 15 min, at bottom of the monovette approximately 0.7 mm was PRP and the upper rest was platelet-poor plasma (PPP), which was collected separately. PRP was then vortexed for 20s and final preparation of PRP transferred into an application injector.

Surgical procedure

Test and control sites were determined via the flip of a coin[22] by single researcher and always received surgery in the same surgical session. All surgical procedures were performed by a single operator (P.G.). Both patient and investigator who takes care of periodontal examinations were blinded about sites of test and control.

After local anesthesia (Ultracaine D-SVR, Hoechst A.G., Turkey) an intra-sulcular incision was made at the buccal aspect of the involved tooth. Two horizontal incisions were made at right angles to the adjacent interdental papillae, at the level of the CEJ, a split-thickness flap was extended apically, releasing the tension and favoring the coronal positioning of the flap. The epithelium on the adjacent papillae was stripped away. The root surface was instrumented and washed with sterile saline. ADM was adapted after rehydration in sterile saline, according to the manufacturer's instructions. The graft was trimmed to a shape and size designed to cover the root surface and the surrounding bone. The coronal lateral borders of the ADM were sutured to the lingual gingival tissue with resorbable polyglycolide-co-lactide sutures (Pegelak 5/0, Dogsan, Trabzon, Turkey). The flap was coronally positioned and sutured to cover the allograft completely. The sites of both CAF + ADM and CAF + ADM + PRP groups received identical surgical treatment including ADM placement, except ADM was hydrated in PPP,[5] and the PRP was applied to surgical sites prior to final suturing in CAF + ADM + PRP group.

Post-surgical care

All patients were instructed to discontinue brushing around the surgical sites for 4 weeks after the surgery. During this post-surgical period, the plaque control was achieved with serum physiologic rinse used twice a day. Non-steroidal anti-inflammatory drugs were prescribed as analgesics to control postoperative discomfort. The sutures were removed after 14 days. One month after the surgeries, the patients were instructed to resume mechanical tooth cleaning of the treated areas using a soft toothbrush and a roll-technique. All patients were recalled for a professional prophylaxis and plaque control, once a week during the first month, fortnightly until the third month, once a month until the end of the study.

Statistical analysis

Data were reported as mean ± standard deviation (SD). Statistical analysis was performed by statistical software (SPSS Inc., Chicago, IL, USA). The Wilcoxon test was used to analyze parameters before and after treatment within each group. The Mann–Whitney U was used to analyze parameters between test and control groups. All tests were two-sided, and differences were considered statistically significant at $P < 0.05$. Differences in the changes in GRD between maxillary and mandibular teeth were analyzed by the Mann–Whitney U test.

Results

Healing was uneventful for all patients. Four females and eight males with a mean age of 37 ± 14 years (range, 20–67 years) were included in the study. The treated teeth were 26 incisors, 24 canines and 34 first premolars. The both CAF + ADM group and CAF + ADM + PRP group consisted of 19 maxillary and 23 mandibular teeth, respectively (Table 1). Total of 84 MAGRs (42 teeth each in the CAF + ADM + PRP and CAF + ADM groups) were included (Table 2).

A power calculation was done after the completion of the study, assuming the mean clinical attachment gain from baseline to 12 months as a primary outcome variable. This analysis indicated that the sample size of 10 patients afforded 84% statistical power to detect a difference of 1 mm CAL between the treatments, adopting an alpha = 0.05. To allow for possible dropouts, 14 patients were recruited. Two patients who failed to return for all postoperative visits were exited from the study.

GRD and GRW reductions at postsurgical 3, 6 and 12 months were significant in both groups, compared to baseline ($P < 0.05$) (Table 3) (Figs. 1 and 2). On the other hand, when groups compared to each other, test group revealed significantly enhanced GRD and GRW reductions at 6 and 12 months after surgery, compared to the test group ($P < 0.05$).

The mean WKT of the test and control groups were 1.2 mm and 1.3 mm at baseline, respectively (Table 3). WKT

Table 3 Clinical parameters at baseline, 3, 6 and 12 months postoperatively.

Clinical Parameters	CAF + ADM + PRP	CAF + ADM
Gingival Recession Depth (GRD) (mm)		
Baseline	3.7 ± 0.4^a	3.7 ± 0.4
3 months	0.5 ± 0.2^b	0.6 ± 0.2^b
6 months	$0.7 \pm 0.1^{b,d}$	$0.9 \pm 0.3^{b,d}$
12 months	$0.8 \pm 0.1^{b,d}$	$1.1 \pm 0.4^{b,d}$
Gingival Recession Width (GRW) (mm)		
Baseline	3.8 ± 0.3	3.8 ± 0.3
3 months	0.5 ± 0.2^b	0.6 ± 0.2^b
6 months	$0.7 \pm 0.2^{b,d}$	$0.9 \pm 0.2^{b,d}$
12 months	$0.8 \pm 0.2^{b,d}$	$1.1 \pm 0.3^{b,d}$
Width of Keratinized Tissue (WKT) (mm)		
Baseline	1.2 ± 0.3	1.2 ± 0.4
3 months	4.0 ± 0.4^b	3.9 ± 0.4^b
6 months	3.8 ± 0.4^b	3.6 ± 0.5^b
12 months	3.8 ± 0.5^b	3.6 ± 0.5^b
Creeping Attachment (CRA) (mm)		
3 months	3.2 ± 0.4^d	3.1 ± 0.2^d
6 months	3.0 ± 0.3	2.8 ± 0.1
12 months	2.9 ± 0.3^c	2.6 ± 0.5
Root Coverage (RC) (%)		
3 months	86.5 ± 5.7	82.3 ± 7.5
6 months	80.9 ± 3.5^b	75.5 ± 9.7^b
12 months	$77.9 \pm 4.4^{b,d}$	$69.4 \pm 13.1^{b,d}$

[a] Data shown as mean ± standard deviation (SD).
[b] Statistically significant difference compared to baseline ($P < 0.05$), Wilcoxon Test.
[c] Statistically significant difference compared to 3rd. month ($P < 0.05$), Wilcoxon Test.
[d] Statistically significant difference between groups ($P < 0.05$), Mann Whitney U Test.

increased significantly in both groups at 3, 6 and 12 months postoperatively ($P < 0.05$). However, WKT differences between the groups were insignificant.

At 3 months, the test group showed a greater CRA gain ($P < 0.05$). The CRA alteration for control group appeared insignificant between 6 and 12 months, on the other hand CRA increment in test group found significant between 6 and 12 months.

Both treatments resulted in significant improvements of RC (Table 3). At 3 months, RC% were 86.5% (73.1%–94.2%) and 82.3% (68.6%–94.7%), respectively, for test and control groups. At 6 months, RC% were 80.9% (75.9%–86.6%) in the test and 75.5% (57.2%–90.8%) in the control group. At the 12-month follow-up, RC% was 77.9% for test group (69.1%–82.6%) and 69.4% (45.5%–90.8%) for the control group. The comparisons of RC between groups did not show significant difference at 3 and 6 months, while RC at 12 months was statistically higher in test group ($P < 0.05$).

There were no significant differences in terms of PI and GI in both groups (Table 4). In both groups, PD increased throughout the study. PD demonstrated significant difference at 3, 6 and 12 months with regards to baseline in test group ($P < 0.05$). In control group, significant difference was observed after 6 and 12 months compared to baseline ($P < 0.05$). No significant differences between the groups were observed

Table 2 Distribution of operation sites according to the multiple adjacent teeth.

Patient	CAF + ADM + PRP	CAF + ADM
1	14-13-12-11	24-23-22-21
2	13-12-11	23-22-21
3	44-43-42-41	34-33-32-31
4	44-43-42-41	34-33-32-31
5	45-44-43-42	35-34-33-32
6	15-14-13	25-24-23
7	15-14-13	25-24-23
8	15-14-13	25-24-23
9	45-44-43-42-41	35-34-33-32-31
10	45-44-43	35-34-34
11	45-44-43	35-34-33
12	13-12-11	23-22-21
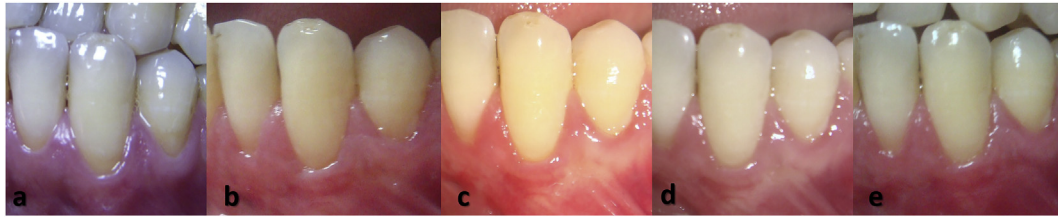
Figure 1 Baseline **(a)**, and postsurgical 3-month **(b)**, 6-month **(c)**, 9-month **(d)** and 12-month **(e)** results for CAF + ADM + PRP group.
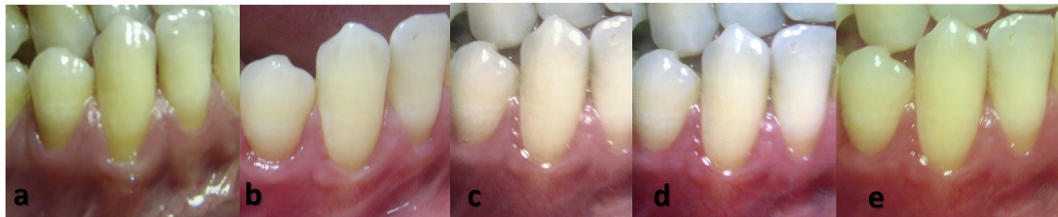
Figure 2 Baseline **(a)**, and postsurgical 3-month **(b)**, 6-month **(c)**, 9-month **(d)** and 12-month **(e)** results for CAF + ADM group.

Table 4 Clinical Parameters at baseline, 3, 6 and 12 months postoperatively.

Clinical Parameters	CAF + ADM + PRP	CAF + ADM
Plaque Index (PI)		
Baseline	0.2 ± 0.1^a	0.1 ± 0.1
3 months	0.2 ± 0.1	0.1 ± 0.09
6 months	0.1 ± 0.1	0.2 ± 0.3
12 months	0.1 ± 0.1	0.1 ± 0.1
Gingival Index (GI)		
Baseline	0.1 ± 0.1	0.1 ± 0.1
3 months	0.1 ± 0.1	0.1 ± 0.1
6 months	0.1 ± 0.1	0.1 ± 0.1
12 months	0.1 ± 0.1	0.1 ± 0.1
Probing Depth (PD) (mm)		
Baseline	1.3 ± 0.2^c	1.2 ± 0.4^c
3 months	1.4 ± 0.2^b	1.4 ± 0.1
6 months	1.5 ± 0.1^b	1.5 ± 0.2^b
12 months	1.6 ± 0.2^b	1.6 ± 0.2^b
Clinical Attachment Level (CAL) (mm)		
Baseline	5.0 ± 0.4	4.8 ± 0.4
3 months	1.9 ± 0.2^b	1.9 ± 0.3^b
6 months	2.2 ± 0.3^b	2.4 ± 0.3^b
12 months	$2.3 \pm 0.2^{b,c}$	$2.7 \pm 0.4^{b,c}$

[a] Data shown as mean $\pm$ SD.
[b] Statistically significant difference compared to baseline ($P < 0.05$), Wilcoxon Test.
[c] Statistically significant difference between groups ($P < 0.05$), Mann Whitney U Test.

Table 5 Reduction of gingival recession depths (GRD) among maxillary and mandibular teeth at 3, 6 and 12-month follow-ups according to groups.

Groups	Maxillary Teeth	Mandibular Teeth
CAF + ADM + PRP		
3 months	3.36 ± 0.04^a	3.13 ± 0.34
6 months	3.13 ± 0.16^b	2.87 ± 0.25^b
12 months	$3.02 \pm 0.06^{b,c,d}$	$2.82 \pm 0.37^{b,d}$
CAF + ADM		
3 months	3.08 ± 0.09	2.36 ± 0.29
6 months	2.82 ± 0.07^b	2.80 ± 0.46^b
12 months	2.61 ± 0.03^b	2.36 ± 0.29^b

[a] Data shown as the mean $\pm$ SD.
[b] $P < 0.05$ compared to 3rd month, by Wilcoxon Signed Test.
[c] $P < 0.05$ compared to 6th month by Wilcoxon Signed Test.
[d] Statistically significant difference between groups ($P < 0.05$), Mann Whitney U Test.

periods, regardless of the treatment technique. At the same time, GRD reduction averaged 3.02 mm for maxillary teeth and 2.82 mm for mandibular teeth treated with CAF + ADM + PRP, and 2.61 mm for maxillary teeth and 2.36 mm for mandibular teeth treated with CAF + ADM at 12 months; the differences were significant between the maxillary and the mandibular teeth. At the end of 12 months, the mean recession depth reduced from 3.71 mm to 0.89 mm in maxillary teeth and 3.60 mm−1.01 mm in mandibular teeth regardless of treatment modalities. Greater change in GRD was found in the maxillary teeth of test group compared to the mandibular teeth of control group, however, the difference was not significant. Test group showed significant difference between 3, 6 and 12 months in terms of GRD in maxillary teeth ($P<0.05$), however, there were no significant improvement between 6 months and 12 months' interval in mandibular teeth of control group.

at 3 and 6 months postoperatively in terms of CAL, however the control group showed a greater CAL when compared with the test group, at 12 months ($P < 0.05$) (Table 4).

Based on tooth location, maxillary versus mandibular, GRD was also assessed (Table 5). GRD differences between maxillary and mandibular teeth were significant for all

Discussion

The aim of this 12-month, randomized, split mouth clinical investigation was to determine whether PRP plus an underlying ADM graft would provide improved clinical results when compared to ADM alone. To date, only few studies[5,15] have evaluated the combination of ADM and PRP as alternative root coverage method for treatment of single Miller Class I and II gingival recessions. However, no randomized trials have compared the results of a CAF + ADM + PRP treatment with CAF + ADM treatment in MAGRs located bilaterally.

In the present study at the postoperative 3rd and 12th month, GRD reductions were 3.2 mm and 2.9 mm for CAF + ADM + PRP group, along with 2.9 mm and 2.6 mm for the CAF + ADM group, respectively. Similarly, Shepherd et al.[5] treated single gingival recessions with coronally positioned tunnel (CPT)+ADM alone or with PRP, and achieved 2.9 mm GRD reduction after 4 months with CPT + ADM + PRP. Moreover, according to our data, CAF + ADM + PRP group also revealed significantly better GRW reduction when compared to CAF + ADM group at the end of the 12 month. A previous pilot study[15] reported that PRP + CAF application for root coverage did not improve GRW reduction outcomes, when compared with CAF alone. It can be suggested that CAF + ADM + PRP might be an effective treatment combination for improving of GRD and GRW in the treatment of MAGRs.

In our study, both procedures successfully resulted with significant WKT increases in all evaluation periods, compared to baseline, however, no significant differences were found between two groups. These results were in agreement with the results of previous studies[5,15,23] in which the WKT increase showed no significant improvement with the use of PRP.

Borghetti and Gardella[22] documented that CRA gain may continue for 1 year post operatively. Previously, nonsignificant CRA alterations were reported in CAF + ADM treated cases from 2 to 12 months[24] and 2—6 months.[7] It was reported that 6-month postoperative measurement period is sufficient to evaluate the stability of the gingival margin after a CAF.[25] Also, stable gingival margin levels were reported up to 1 year after CAF procedure.[26,27] In our study, significant CRA increase was seen in both groups at 6th and 12th month, compared to 3rd month results.

In the present study, RC data were almost identical at first 6 months in both groups. However, CAF + ADM + PRP group demonstrated significantly higher RC at the end of the 12th month. CAF + ADM + PRP significantly improved CRA and RC according to postoperative 12th month data, when compared with CAF + ADM alone (2.9 versus 2.6 mm, 77.9 versus 69.4 mm, respectively.) This result was in line with the study by Shepherd et al. in which 78% RC was obtained by CPT + ADM + PRP treatment. According to present data, PRP might improve the potential of CAF + ADM treatment in terms of providing superior stability of coronally repositioned gingival margin for 12-month follow-up period.

Previous studies evaluated the outcomes of root-coverage using ADM with the tunneling technique,[6] with CAF[7,24,27] and application of PRP + CAF.[15] Papageorgakopoulos et al.[6] found 78% RC in 12 defects using ADM with tunnel technique, at 4-month follow-up; Henderson et al.[24] found 95% RC in 10 defects at the 12th month; Woodyard et al.[7] found 99% RC in 12 defects at the 6th month; Tal et al.[27] found 89% RC in 7 defects using ADM + CAF at 12 months and Huang et al.[15] found 81% coverage in 23 patients with application of PRP + CAF at 6-month follow-up. The RC% obtained in this study was different from the most of the studies reported. These various results can be explained by the differences in initial recession dimensions, evaluation periods, sample size, surgical procedure and number of multiple adjacent teeth. For instance, Woodyard et al.[7] have shown that CAF + ADM significantly increased gingival thickness and improved RC at 6 months with regards to baseline. However, unlike our study design, in that study, root surfaces were conditioned with tetracycline before surgery and systemic doxycycline were prescribed to subjects postoperatively. In our study, root surface conditioning was not applied and both antibiotics and chlorhexidine was not used during post-operation period, so that the PRP's actual contribution to the ADM could be found independent of other variables.

Our data showed similar alterations for PD in both groups in all the follow-up periods, in agreement with other studies.[28,29] However, for the CAL, significant difference was favoring the CAF + ADM + PRP group.

Besides PRP, some other autogenous platelet preparations derived from venous blood was also studied for treatment of gingival recessions. In a recent study[30] wherein leukocyte- and platelet-rich fibrin (L-PRF), has been compared to CTG for the treatment of MAGRs, RC% were found 76.6% and 77.4%, respectively, at the end of 1 year and thereby, they suggested that L-PRF could serve as an alternative for CTG. In that study, 8 pairs of recession sites comprising of 2 adjacent teeth and 2 pairs of 3 adjacent teeth was included.[30] In our study, the treatment of 7 pairs of 3 adjacent teeth, 4 pairs of 4 adjacent teeth and 1 pair of 5 adjacent teeth, showed that combined application of ADM and PRP increases RC% at the end of first year. In a study applying modified-CAF alone or together with PRF for the treatment of 18 pairs of Miller Class I or II MAGRs during 6 months, it was revealed that application of modified-CAF + PRF provided lower RC% compared to modified-CAF alone, but increased gingival thickness.[3] Within the scope of the aforementioned study, a 0.5-mm-thick PRF located at the flap margins. The lower RC% in modified-CAF + PRF group might be attributed to the restricted collateral circulation under the flap due to thickness of PRF and thereby, reduction of revascularization and healing. Compared to study of Aroca et al.,[3] higher RC% of 80.9% was obtained by ADM + PRP group in our study; whereas it was 75.5% for ADM alone at the end of 6th month. Our data revealed better RC% for CAF + ADM + PRP group, which may be explained with absence of extra thickness with PRP, compared to PRF. Considering ADM's thickness is already 0.25—0.59 mm, even approximately 0.5 mm of PRF may interfere with the results, particularly at whom with thin gingival biotype, who are also more prone to gingival recessions. As it is specified by Hwang et al., the interposition of PRF may restrict the collateral circulation, which is essential for a thin flap to revascularize and heal.[31] The fact that the compromised collateral circulation under the flap due to thickness of PRF and hence decreased revascularization and healing is of greater importance in cases where

number of adjacent teeth to be treated. For example, in the present study, MAGR treatment of one patient with bilateral MAGRs each located to 5 teeth, GRD and GRW of the test group reduced by 3 and 2.9 mm, respectively, at the end of 1 year; however, as for the control group, reduction in the both measures was limited to 2.8 mm.

The present study was also designed to test the difference between maxillary and mandibular sites, where 50% of the sites were maxillary and 50% were mandibular. There were no significant differences in the initial GRD between two arches. Although GRD reduced significantly in both arches, in test group, significantly more GRD reduction was observed in maxillary teeth. The difference between two arches in test group may be due to synergistic interaction of passive coronal positioning/displacement seen in the maxilla and additional effects of PRP. The differences may also depend on the soft tissue defects related factors including the width and height of interdental soft tissue, the vestibular depth, the presence of frenenuli. Frenulums are known to have greater effect across the mandible.[32] In the study by Shepherd et al.,[5] where the tunnel technique used for recession treatment, difficulty of achieving the same degree of flap release in mandibular sites, as in maxillar sites, was noted. Due to the increased flap tension in mandibular sites, they reported that the CAF for maxillary sites seemed to be a highly predictable procedure, whereas for mandibular sites the outcome was much less predictable.

In the present study, the ADM was covered by gingival flap, which was prepared with vertical incisions as well as intra-sulcular incisions. In this way the flap tension decreased which enhanced the likelihood of recession coverage. Pini-Prato et al.[26,33] reported that sites with higher flap tension were associated with lower GRD reductions. On the other hand, vertical incisions were allowed to cover ADM completely with vital tissues such as gingival flap and PRP. Because it was suggested that the non-vital ADM allograft lacked the capability of directing cyto-differentiation of the covering epithelium, care was taken not to leave exposed ADM, in view of its ability to revascularize only when in direct contact with vital tissue.[34–36] The present study include application of ADM + PRP combination on the basis of the assumption that outcomes obtained by ADM might be improved and their long-term sustainability might be ensured in combination with PRP. Main purpose of PRP application is to increase platelet derived growth factors, such as PDGF, TGF-beta, and IGF-I, in the treatment site which may contribute to the wound healing and to facilitate healing process.[37–40] In the scope of present study, it was observed that soft tissue healing was facilitated through usage of ADM + PRP combination, pre- and post-operation bleeding decreased and ADM was positioned easier in existence of PRP and thereby, stabilization was increased.

When interpreting the present results, patient selection criteria and the study design should also be evaluated for better understanding of the study outputs. Although smokers were included in the similar previous studies,[41,42] the participants of the present study consist of only non-smokers, due to the fact that smoking has long-term effects. Smoking status may negatively influence the expected results linked to RC% of MAGRs. Previously, a systematic review by Chambrone et al.[43] demonstrated that non-smokers may obtain 17.5% additional RC% than

smokers when patients were treated with a CTG. It is well known that smoking has negative effects on vascularization of periodontal tissues and wound healing. The long-term stability of GRs treated with CTG + CAF was assessed within a sample of 30 non-smoking and 25 smoking patients, for 36 months.[44] At the 12-month evaluation, the authors reported mean RC of 92.6% for non-smokers and 89.0% for smokers; however, after 36 months, these rates decreased to 81.5% and 68%, respectively.

Appropriate subgroup selection is important to obtain a powerful estimation of treatment effect and right conclusion. The present study designed as a split-mouth study to exclude the influence of individual patient characteristics. In our study design, each patient received both test and control treatment at the same therapy session, at the right and the left side of the mouth. This study included multiple adjacent symmetric recessions located at both maxillary and mandibular arches showing a fitted equation in the number of operated teeth. In the present study no root conditioning, chlorhexidine and antibiotics were used because it is not possible to determine to what extent and under what conditions they may have contributed to post-operative results. Application of root conditioners, administration of chlorhexidine and antibiotics can be accepted as additional steps during periodontal therapy. This approach may allow providing valuable insight based on the precise clinical role of PRP with the goal of treating MAGRs, and thus, increasing the clinical relevance of the results.

Within the limit of this study, the additional application of PRP to the CAF + ADM treatment of MAGRs was effective in terms of GRD and GRW reduction and RC%, with significant difference between the groups. The results clearly indicate that the recession coverage obtained with CAF + ADM + PRP may be successfully maintained over a 1-year follow-up. Our data revealed significant differences between groups in GRD, GRW, CRA and RC% parameters, which favored the CAF + ADM + PRP group. In conclusion, our results suggest that the combination of ADM and PRP, along with CAF, as an alternative root coverage procedure, is a suitable option for the treatment of MAGRs.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

References

1. Aroca S, Keglevich T, Nikolidakis D, et al. Treatment of class III multiple gingival recessions: a randomized-clinical trial. *J Clin Periodontol* 2010;37:88–97.
2. Hofmanner P, Alessandri R, Laugisch O, et al. Predictability of surgical techniques used for coverage of multiple adjacent gingival recessions—A systematic review. *Quintessence Int* 2012;43:545–54.

3. Aroca S, Keglevich T, Barbieri B, Gera I, Etienne D. 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. *J Periodontol* 2009;80:244—52.

4. Livesey SA, Herndon DN, Hollyoak MA, Atkinson YH, Nag A. Transplanted acellular allograft dermal matrix. Potential as a template for the reconstruction of viable dermis. *Transplantation* 1995;60:1—9.

5. Shepherd N, Greenwell H, Hill M, Vidal R, Scheetz JP. Root coverage using acellular dermal matrix and comparing a coronally positioned tunnel with and without platelet-rich plasma: a pilot study in humans. *J Periodontol* 2009;80: 397—404.

6. Papageorgakopoulos G, Greenwell H, Hill M, Vidal R, Scheetz JP. Root coverage using acellular dermal matrix and comparing a coronally positioned tunnel to a coronally positioned flap approach. *J Periodontol* 2008;79:1022—30.

7. Woodyard JG, Greenwell H, Hill M, Drisko C, Iasella JM, Scheetz J. The clinical effect of acellular dermal matrix on gingival thickness and root coverage compared to coronally positioned flap alone. *J Periodontol* 2004;75:44—56.

8. Shulman J. Clinical evaluation of an acellular dermal allograft for increasing the zone of attached gingiva. *Pract Periodontics Aesthet Dent* 1996;8:201—8.

9. Callan DP, Silverstein LH. Use of acellular dermal matrix for increasing keratinized tissue around teeth and implants. *Pract Periodontics Aesthet Dent* 1998;10:731—4.

10. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *Int J Oral Maxillofac Implants* 1999;14:529—35.

11. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998;85:638—46.

12. Lynch SE, Colvin RB, Antoniades HN. Growth factors in wound healing. Single and synergistic effects on partial thickness porcine skin wounds. *J Clin Invest* 1989;84:640—6.

13. Lacoste E, Martineau I, Gagnon G. Platelet concentrates: effects of calcium and thrombin on endothelial cell proliferation and growth factor release. *J Periodontol* 2003;74: 1498—507.

14. Bashutski JD, Wang HL. Role of platelet-rich plasma in soft tissue root-coverage procedures: a review. *Quintessence Int* 2008;39:473—83.

15. Huang LH, Neiva RE, Soehren SE, Giannobile WV, Wang HL. The effect of platelet-rich plasma on the coronally advanced flap root coverage procedure: a pilot human trial. *J Periodontol* 2005;76:1768—77.

16. Miller Jr PD. A classification of marginal tissue recession. *Int J Periodontics Restor Dent* 1985;5:8—13.

17. Silness J, Loe H. Periodontal disease in pregnancy. Ii. Correlation between oral hygiene and periodontal condtion. *Acta Odontol Scand* 1964;22:121—35.

18. Loe H. The gingival index, the plaque index and the retention index systems. *J Periodontol* 1967;38(Suppl):610—6.

19. de Queiroz Cortes A, Sallum AW, Casati MZ, Nociti Jr FH, Sallum EA. A two-year prospective study of coronally positioned flap with or without acellular dermal matrix graft. *J Clin Periodontol* 2006;33:683—9.

20. Bethaz N, Romano F, Ferrarotti F, Mariani GM, Aimetti M. A mucogingival technique for the treatment of multiple recession defects in the mandibular anterior region: a case series with a 2-year follow-up. *Int J Periodontics Restor Dent* 2014; 34:345—52.

21. Ozdemir B, Okte E. Treatment of intrabony defects with beta-tricalciumphosphate alone and in combination with platelet-rich plasma. *J Biomed Mater Res B Appl Biomater* 2012;100: 976—83.

22. Borghetti A, Gardella JP. Thick gingival autograft for the coverage of gingival recession: a clinical evaluation. *Int J Periodontics Restor Dent* 1990;10:216—29.

23. Cheung WS, Griffin TJ. A comparative study of root coverage with connective tissue and platelet concentrate grafts: 8-month results. *J Periodontol* 2004;75:1678—87.

24. Henderson RD, Greenwell H, Drisko C, et al. Predictable multiple site root coverage using an acellular dermal matrix allograft. *J Periodontol* 2001;72:571—82.

25. Cheng YF, Chen JW, Lin SJ, Lu HK. Is coronally positioned flap procedure adjunct with enamel matrix derivative or root conditioning a relevant predictor for achieving root coverage? A systemic review. *J Periodontal Res* 2007;42:474—85.

26. Pini Prato G, Pagliaro U, Baldi C, et al. Coronally advanced flap procedure for root coverage. Flap with tension versus flap without tension: a randomized controlled clinical study. *J Periodontol* 2000;71:188—201.

27. Tal H, Moses O, Zohar R, Meir H, Nemcovsky C. Root coverage of advanced gingival recession: a comparative study between acellular dermal matrix allograft and subepithelial connective tissue grafts. *J Periodontol* 2002;73:1405—11.

28. Trombelli L, Scabbia A, Tatakis DN, Calura G. Subpedicle connective tissue graft versus guided tissue regeneration with bioabsorbable membrane in the treatment of human gingival recession defects. *J Periodontol* 1998;69:1271—7.

29. Wang HL, Bunyaratavej P, Labadie M, Shyr Y, MacNeil RL. Comparison of 2 clinical techniques for treatment of gingival recession. *J Periodontol* 2001;72:1301—11.

30. Tunaliota M, Ozdemir H, Arabaciota T, Gurbuzer B, Pikdoken L, Firatli E. Clinical evaluation of autologous platelet-rich fibrin in the treatment of multiple adjacent gingival recession defects: a 12-month study. *Int J Periodontics Restor Dent* 2015;35: 105—14.

31. Hwang D, Wang HL. Flap thickness as a predictor of root coverage: a systematic review. *J Periodontol* 2006;77:1625—34.

32. Placek M, Skach M, Mrklas L. Problems of the labial frenum attachment in periodontics. II. Attempt to determine the resistance of periodontium to the influence of individual types of the labial frenum attachment. *Cesk Stomatol* 1974;74:401—6.

33. Pini-Prato GP, Cairo F, Nieri M, Franceschi D, Rotundo R, Cortellini P. Coronally advanced flap versus connective tissue graft in the treatment of multiple gingival recessions: a split-mouth study with a 5-year follow-up. *J Clin Periodontol* 2010;37:644—50.

34. Wainwright DJ. Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. *Burns* 1995;21:243—8.

35. Rhee PH, Friedman CD, Ridge JA, Kusiak J. The use of processed allograft dermal matrix for intraoral resurfacing: an alternative to split-thickness skin grafts. *Arch Otolaryngol Head Neck Surg* 1998;124:1201—4.

36. Wainwright D, Madden M, Luterman A, et al. Clinical evaluation of an acellular allograft dermal matrix in full-thickness burns. *J Burn Care Rehabil* 1996;17:124—36.

37. Camelo M, Nevins ML, Schenk RK, Lynch SE, Nevins M. Periodontal regeneration in human Class II furcations using purified recombinant human platelet-derived growth factor-BB (rhPDGF-BB) with bone allograft. *Int J Periodontics Restor Dent* 2003;23:213—25.

38. Giannobile WV, Finkelman RD, Lynch SE. Comparison of canine and non-human primate animal models for periodontal regenerative therapy: results following a single administration of PDGF/IGF-I. *J Periodontol* 1994;65:1158—68.

39. Howell TH, Fiorellini JP, Paquette DW, Offenbacher S, Giannobile WV, Lynch SE. A phase I/II clinical trial to evaluate a

combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. *J Periodontol* 1997;68:1186—93.

40. Nevins M, Camelo M, Nevins ML, Schenk RK, Lynch SE. Periodontal regeneration in humans using recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and allogenic bone. *J Periodontol* 2003;74:1282—92.

41. Pini Prato GP, Baldi C, Nieri M, et al. Coronally advanced flap: the post-surgical position of the gingival margin is an important factor for achieving complete root coverage. *J Periodontol* 2005;76:713—22.

42. Erley KJ, Swiec GD, Herold R, Bisch FC, Peacock ME. Gingival recession treatment with connective tissue grafts in smokers and non-smokers. *J Periodontol* 2006;77:1148—55.

43. Chambrone L, Chambrone D, Pustiglioni FE, Chambrone LA, Lima LA. The influence of tobacco smoking on the outcomes achieved by root-coverage procedures: a systematic review. *J Am Dent Assoc* 2009;140:294—306.

44. Jankovic S, Aleksic Z, Dimitrijevic B, Camargo P, Kenney B, Lekovic V. Impact of interleukin 1 gene polymorphism and smoking on long-term stability following gingival recession treatment. *Int J Periodontics Restor Dent* 2013;33:e16—23.