Recombinant human Platelet-Derived Growth Factor-Soaked Xenogeneic Collagen Matrix versus Placebo for the treatment of Multiple Adjacent Gingival Recessions: A triple-blinded, randomized, placebo-controlled trial

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Abstract

Aim: To test the efficacy of recombinant human platelet-derived growth factor-BB (rhPDGF) in addition to a xenogeneic collagen matrix (XCM) for the treatment of multiple adjacent gingival recession defects (MAGRs) in combination with the coronally advanced flap (CAF).

Materials and Methods: Thirty patients were enrolled in this triple-blind placebo-controlled randomized trial and treated with CAF + XCM + rhPDGF (test), or CAF + XCM + vehicle (saline solution, control). The primary outcomes included mean root coverage (mRC) and complete root coverage (CRC). Gain in gingival thickness (GT) and keratinized tissue width (KTW) as well as volumetric changes and ultrasonographic (US) tissue perfusion variations were assessed. Linear mixed-effect regression models were used for statistical comparison.

Results: At 6 months, the mRC of the test and control groups were 90.05%, and 84.32% respectively ($p<0.05$). A significant gain in GT was consistently observed for both treatment arms, and more so for the test group ($p<0.05$). The test group showed higher 3D volume gain and linear US soft tissue thickness. US revealed significant higher tissue perfusion analysis at 2 weeks for the rhPDGF-treated sites.

Conclusion: rhPDGF can enhance the outcomes of MAGRs treated with CAF + XCM. Greater volume and esthetic outcomes were also observed in the group that received rhPDGF as compared to vehicle (ClinicalTrials.gov NCT04462237).

1. Introduction

Gingival recession is a common condition that affects a significant portion of the population [1, 2]. Studies have demonstrated that among the variety of treatments available for root coverage, autogenous connective tissue graft (CTG)-based techniques are the most effective and predictable [3, 4]. While most of the evidence on root coverage outcomes with the CTG or other techniques comes from treatment of isolated recession defects, gingival recession is more often a generalized condition [2, 5]. Therefore, it is not surprising that CTG substitutes, such as allogeneic dermal grafts and collagen matrices, have progressively gained popularity in the clinical arena for reducing patient morbidity, and due to their unlimited resources, making them particularly indicated for the treatment multiple adjacent gingival recessions (MAGRs) [6].

A novel porcine, porous collagen matrix has recently been introduced for soft tissue augmentation [7-9]. This xenogeneic collagen matrix (XCM) has also been referred to as a volume-stable collagen matrix due to its properties of maintaining a stable augmented volume [7-9]. It is reasonable to assume that this novel XCM may also serve as a scaffold for the ingrowth of cells following growth factor-mediated root coverage procedures. An ex vivo study showed that when soaked with recombinant human platelet-derived growth factor-BB (rhPDGF), this novel cross-linked XCM, can serve as a porous scaffold and increase cellular population and metabolic activity in the matrix [10].

rhPDGF is known as a potent mitogen for fibroblasts and periodontal ligament cells [11, 12]. It promotes angiogenesis by stimulating macrophages to synthesize fibroblast growth factors and transforming growth factor beta. rhPDGF can also accelerate the rate of wound healing by enhancing fibroblast recruitment and activation and by increasing the wound breaking strength[11, 12]. rhPDGF with beta-tricalcium phosphate has been found to promote regeneration of Sharpey's fibers, new cementum and new bone in teeth with isolated gingival recessions [13, 14]. We speculate that rhPDGF can also enhance the properties of XCM in a clinical setting. Therefore, the aim of the present study was to investigate the effect of rhPDGF in combination with XCM for the treatment of MAGRs.

2. Materials and Methods

2.1 Study design and trial registration

The present study was designed as a triple-blind, parallel-arm, randomized, placebo-controlled clinical trial to test the efficacy of rhPDGF in combination with XCM (CAF + XCM + rhPDGF as the test group), versus XCM alone (CAF + XCM, as control) for the treatment of MAGRs.

The trial was registered at ClinicalTrial.gov (NCT04462237) and follows the CONSORT statement [15, 16]. The study protocol was approved by the Institutional Review Board of the University of Michigan Medical School (HUM00177214), in accordance with the Declaration of Helsinki of 1975, revised in Tokyo in 2004. To reduce potential risks related to protection of rights and well-beings of participants and to ensure adequate quality in patient enrollment, intervention, data collection and compliance with the protocol, all phases of the clinical trial were observed by an independent study monitor (L.K.).

2.2 Participants

Participants were recruited based on the following inclusion criteria:

i) Periodontally and systemically healthy adults (age ≥ 18 years) presenting with at least 2 MAGRs classified as recession type 1 (RT1)[17], associated with dental hypersensitivity or esthetic concerns; ii) self-reported smoking ≤ 10 cigarettes/day; iii) full-mouth plaque score and full-mouth bleeding score ≤ 20%; iv) presence of a least 2 mm depth on either recessions, and v) patients being able to maintain good oral hygiene.

The exclusion criteria included: i) compromised general health, ii) pregnancy or attempting to get pregnant (self-reported), iii) untreated periodontal disease, iv) persistence of uncorrected gingival trauma from toothbrushing, v) presence of severe tooth malposition, rotation or super-eruption, vi) previous periodontal plastic surgery at the experimental sites, ix) known allergy to collagen-based medical products.

2.3 Interventions

Eligible patients received a session of dental prophylaxis, including oral hygiene instructions aimed at eliminating possible traumatic toothbrushing habits at least 1 month before the surgery. The intervention consisted of CAF with a XCM, either saturated with a sterile saline solution (as placebo for the control group) or with rhPDGF (test group) (Fig. 1). Based on the location and distribution of the MAGRs, CAF was performed with a trapezoidal or envelope design, with horizontal or rotated papillae, with or without vertical incisions [18-20]. The root surfaces that were exposed to the oral cavity were scaled, planed and chemically conditioned using 24% of EDTA for 2 minutes [21]. For both groups, the XCM was first extraorally trimmed with a 15c blade, based on the characteristics of the recession defects. The matrices were then saturated with a micro-injection needle containing 1.5 cc of the solution that was prepared and provided by another study member through a sealed envelope. All envelopes similarly stated "Research Solution" with the patients' consecutively assigned ID numbers that were also marked on the injection needles. The grafts were left in the dappen dish for 15 minutes [22, 23]. The solution was also applied onto the dried root surfaces before stabilizing the grafts. Simple interrupted sutures (6/0 and 7/0 PGA, AD Surgical, Sunnyvale, USA) engaging the graft and the de-epithelialized anatomical papillae were performed for stabilizing the XCM at the recipient bed, approximately at the level of the CEJ or 1 mm apical. Further stabilization of the graft was also achieved, if necessary, with additional mattress sutures apical to the XCM, through engaging the periosteum. The flap was then coronally advanced and stabilized approximately 2 mm above the cemento-enamel junction with sling sutures and simple interrupted sutures (6/0 and/or 7/0 polypropylene [Ethicon, Johnson & Johnson, Somerville, USA] or [AD Surgical, Sunnyvale, USA)]) at the level of the papillae, completely covering the XCM.

Patients were prescribed Amoxicillin (500 mg 3 times a day for 7 days), Ibuprofen (600 mg every 4-6 hours for the first 3 days, followed by its prescription as needed) and Chlorhexidine mouth rinse (0.12% twice daily for one minute for 14 days). The sutures were removed two weeks after the surgical procedure. Patients were instructed to resume mechanical tooth brushing at the operated area using an extra-soft bristle toothbrush. Patients were recalled at 1 week, 2 weeks, 1 month, 3 months, and 6 months after the surgery.

2.4 Outcomes

The primary outcome of the study was to assess the effect of rhPDGF on the following parameters, after 6 months of healing time on the mean root coverage (mRC) and the frequency of complete root coverage (CRC).

The secondary outcomes that were analyzed and compared within the two groups included:

1) Gingival thickness (GT) gain
2) Keratinized tissue width (KT) gain
3) Esthetic score, using the Root coverage Esthetic Score (RES) system [24].
4) Soft tissue volume change over time, using optical scanning-based tri-dimensional technologies [25]
5) Tissue perfusion analysis over time with non-ionizing real-time ultrasonography [26]
6) Patient-reported outcomes (PROMs)

2.4.1 Clinical measures

The following clinical measurements were performed by a single masked and calibrated examiner (J.M.) at baseline, 3 months, and 6 months after the surgery at the mid-buccal aspect of all treated sites, as previously described [27-29]: i) Recession depth (REC), ii) Probing depth (PD), iii) Clinical attachment level (CAL), iv) Keratinized tissue width (KTW), Gingival thickness (GT), Periodontal soft tissue phenotype using the color-coded

probes (Colorvue probes, Hu-Friedy, Chicago, USA). The esthetic outcomes at 6 months were evaluated using the Root coverage Esthetic Score (RES)[24]. Examiner calibration consisted of two repeated measurements of recession depth (REC) and keratinized tissue width (KTW) in a pool of 10 subjects not participating in the study (K coefficient of 0.89 for REC and of 0.88 for KTW) [30].

2.4.2 STL file acquisition and Volumetric outcome assessment

An intraoral optical scanner (Trios, 3Shape, Denmark) was utilized to generate digital models that were saved as STL files and imported in an image analysis software (GOM Inspect, GOM, Germany). A blinded and pre-calibrated examiner with experience in 3D volumetric analysis (L.M.) performed all the measurements. A semi-automated alignment, based on the selection of reproducible points on the digital models and on a best-fit algorithm, was used to superimpose the STL files. Each time point (1, 3 and 6 months) was superimposed onto baseline, which was used as the reference. The region of interest (ROI) was defined as previously explained [25]. The volumetric outcomes of interest were: i) volume change in mm^3 (Vol), ii) the mean distance between the surface/mean thickness of the reconstructed volume in mm (ΔD), and iii) linear dimensional changes (LD) from 1 to 5 mm from the gingival margin [31-36].

2.4.3 Ultrasonographic outcomes

Real-time ultrasonography was utilized for collecting frame and cine-loop scans at the midfacial and interproximal aspects of the study sites, from which the soft tissue thickness (STT) at 1-, 3-, and 5- mm reference points from the gingival margin were obtained at each time point, using a commercially available software package (Horos™, version 3.3.6, Horos Project) as previously reported [26, 37-40]. Tissue perfusion analysis over time was assessed per tooth at the midfacial and interproximal areas in terms of color doppler velocity and color power changes [26]. Briefly, color velocity (CV) visualizes the speed at which blood flows within the lumens in the field of view, while color power (CP) displays the amount of blood flowing within the lumens in the field of view. The method for computing CV and CP is described in detail in a previous report [26].

2.4.4 Patient-reported outcome measures (PROMs)

PROMs included a questionnaire evaluating patient satisfaction regarding the appearance of the gums at the future surgical site. The questionnaire also included the assessment of the Oral Health Impact Profile (OHIP-14) [20]. This questionnaire was collected at baseline and at the 6-month visit. Patient morbidity, including perceived pain, swelling, painkiller intake, and other symptoms/adverse reactions related to the first 14 days were collected using a novel interactive 3D mobile application (app) (GeoPain, MoxyTech, Ann Arbor, USA) [41].

2.5 Sample size

The study was powered to detect a minimum clinically significant difference in root coverage of 0.5 mm using $\alpha = 0.05$, a power (1- β) of 80%, and a hypothesized within-group sigma of 0.4 mm [28]. Considering possible dropouts, the number of patients were increased by 15% for each arm. On the basis of these data, the minimum number of patients needed to be enrolled in this study was 30 in total, 15 for the test (CAF+ XCM+ rhPDGF), and 15 for the control group (CAF + XCM).

2.6 Randomization

An operator with expertise in biostatistical analyses that was not present at the time of surgical treatments, randomly assigned all patients to either group 1 or 2, using a computer-generated randomization table. The randomizations were performed a total of three times (3 sets), per every 10 recruited individuals prior to their treatment, to counter act possible unequal distribution of potential confounding agents among the allocation, such as major patient characteristics (smoking), baseline characteristics (severity of recession depth at baseline, and location mandible/maxilla). Allocation of patients (identified through their ID number) to groups 1 or 2 in each set, was communicated to the study coordinator, who then with a flip of a coin, would decide which of the two groups would receive the experimental treatment (rhPDGF).

On the day of the surgical procedure, the surgeon would receive a sealed envelope with the patient's ID number and date of the procedure, containing a syringe with 1.5 cc of a clear solution which could have either been sterile saline (for the control group), or rhPDGF (GEM21, Lynch Biologics, Franklin, USA, test group). The test and control envelopes and syringes looked identical. Patients, the surgeon, and other study members were unaware and remained uninformed of the test/control treatment allocation.

2.6.5 Statistical methods & Outcome assessment

The gathered data were entered into a prefabricated spreadsheet, as per patients' ID numbers, without knowledge of test vs. control allocation. Means and standard deviations (SD) were calculated for continuous measures (mRC, KT, STT, Vol, ΔD, LD, etc.). CRC was calculated as the percentage of sites that achieved a complete coverage at 6 months and expressed as a binary outcome.

Linear mixed-effects models were used to assess statistical differences in the level of recession between treatment arms at different time points. The models accounted for repeated measures and correlations induced by multiple sites per patients, and multiple time points. Analysis of Rec was performed longitudinally, initially with the inclusion of Rec baseline to check for successful randomization. To assess the primary outcome (efficacy of rhPDGF relative to Rec change over time) and potential treatment-effect heterogeneity, baseline Rec was included as a fixed-covariate in subsequent models.

The influence of variables relative to Rec change were explored through additional regression models. Confidence intervals (CI) were produced and a *p* value of 0.05 was set for statistical significance. Line charts were used for visualization of continuous means and corresponding SDs of outcomes of interest. The randomization, as to which among the two groups (1 or 2) served as the test sites was revealed at the end of the analysis by the study coordinator (A.O). All analyses were performed by an individual author with experience in biostatistical analyses and study design and methodology (S.B.) with a specified software, who had not taken part in the surgical treatments (RStudio, Version 1.3.959).

3. Results

3.1 Participant flow, baseline data and numbers analyzed

Figure 2 shows the CONSORT flowchart of the study. Thirty subjects, 15 per group were randomized and received the allocated treatments. Each patient received a single (either test or control) treatment consisting of 2-5 MAGRs. All subjects completed the follow-up visits and complied with the study recall appointments. Table 1 shows the patient characteristics and baseline measurements of the study sites within groups.

3.2 Clinical, volumetric, and esthetic outcomes

The XCM graft dimensions did not differ significantly among the test and control groups. The healing was uneventful for all treated sites without any adverse events throughout the study.

Results of the mixed models demonstrated successful randomization among the test and control groups relative to Rec (-0.07 (p=0.78)). It was further observed that Rec baseline moderated the treatment response, and significantly more so for the test sites that were treated with rhPDGF (indicating improvement in the outcomes in case of larger defects), such that for subjects with average baseline recession, those in the test group had smaller Rec at 3 months (0.76 mm, p=0.01) and at 6 months (0.71 mm, p=0.01). Subjects with larger baseline Rec had larger post-treatment recessions, but experienced larger reductions from baseline than those subjects with smaller baseline recessions as well.

Relative to mRC, at the 6-month follow-up, the test group showed a significantly higher mRC (90.50 vs 84.32%, p=0.02), CRC (71.88 vs 54.55%, p=0.1) and GT gain (0.78 vs 0.61 mm, $p<0.05$) compared to the control group, while changes in PD were equal in both groups, as well as the observed CAL gain and changes in KTW (Table 2). The 3D digital analysis revealed a significantly higher Vol, ΔD and LD change at 3 and 5 mm for the test group (Fig. 3). The professional esthetic evaluation using the RES showed a mean score of 9.08 for the test group and 7.41 for the control group ($p<0.05$) (Table 2).

3.3 Linear ultrasonographic outcomes

Table 3 depicts the linear ultrasonographic outcomes at 2 weeks, 3 months and 6 months following the two interventions. At the 6-month time point, both test and control groups resulted in a statistically significant gain in STT at 3- and 5-mm reference points compared to baseline, with the test group exhibiting significantly higher values at 3 and 6 months ($p<0.05$).

3.4 Ultrasonographic tissue perfusion analysis

No differences in tissue perfusion, in terms of CV and CP, were noted between the two groups at baseline.

At the 2-week time point, the test group showed a significantly higher mean CV and mean CP at the midfacial aspect compared to the control group (0.42 vs 0.19 [cm/s] and 0.78 vs 0.41 [arb], respectively, $p<0.01$) (Figure 4). Similarly, a greater mean CV and mean CP were observed for the test sites at the interproximal areas (0.45 vs 0.29 [cm/s] and 0.66 vs 0.46 [arb], respectively, $p<0.01$ for both comparisons). No differences were observed within the groups for the 3- and 6-month time points (Fig. 4).

3.5 Patient-reported outcomes

The test group was associated with significantly lower post-operative pain intensity (VAS) and painkiller consumption then the control group ($p<0.05$). The two groups showed similar outcomes in terms of treatment satisfaction and quality of life ($p<0.05$).

4. Discussion

The present study was designed to evaluate the effect of adjunct rhPDGF on the treatment of MAGRs with the CAF and XCM. The outcomes were assessed through comparison of clinical, volumetric, ultrasonographic and subjective patient-reported measures. Compared to sites allocated to the control group, rhPDGF-treated sites achieved significantly greater mRC and CRC. These findings can be associated to the properties of rhPDGF in enhancing and accelerating the stages of wound healing.

Studies have shown that rhPDGF promotes the migrations of neutrophils and macrophages to the wound site and increases the production of reactive oxygen species, resulting in a shorter inflammatory phase [42-44]. The growth factor has also been shown to accelerate fibroblast proliferation, the production of the extracellular matrix, and blood vessel formation [44-46]. Animal wound models have also shown that the application of rhPDGF significantly increases the rate of re-epithelialization and wound closure [47-49]. It can be assumed that an enhanced initial healing after the root coverage procedure can lead to increased clinical outcomes and higher root coverage by reducing the time in which the flap is not attached to the root surface and the underlying de-epithelialized papillae. Indeed, tensile forces during the early wound healing can violate the integrity of the blood clot that mediates the adhesion of the flap to the surgical site [50-52]. Therefore in our study, it is plausible that rhPDGF may have accelerated the wound healing following CAF + XCM, reducing the initial period during which the surgical site was vulnerable to mechanical trauma, leading to enhanced overall clinical outcomes [52].

Two previous trials by Carney et al. and Parween et al. investigated the clinical benefits of rhPDGF in addition to a soft tissue graft [53, 54]. While the study by Carney et al. did not find differences in the root coverage outcomes of acellular dermal matrix with or without the growth factor [53], Parween and coworkers showed significantly higher mRC and CRC for the group in which CTG was soaked with rhPDGF [54]. Thus, one could assume that the properties of the scaffold material can play a key role on the final outcomes of biologic-mediated approaches and tissue engineering grafting materials [12, 55]. Agis and coworkers showed that the cross-linking and uniform porosity of the novel XCM used in our study can facilitate the migration of fibroblasts. They authors found that loading the scaffold with rhPDGF resulted in an increased cell population and metabolic activity within the scaffold [10], which can also explain our results. In our study, the test sites showed a higher stability of the grafted XCM volume throughout 6-month follow-up. Through the ultrasonographic analysis, we observed that the STT gain at 3 and 6 months was significantly greater for the rhPDGF group. This finding was also consistent with changes in GT assessed with the transgingival piercing and the digital volumetric analysis. The higher volume gain in the sites that received the growth factor could be attributed to the enhanced migration and proliferation of fibroblasts from the adjacent sites promoted by rhPDGF [10].

It has been reported that rhPDGF is also able to downregulate the expression of some matrix metalloproteases (MMPs), such as MMP2 and MMP14 [10], that have been associated with pain and recovery [56-58]. A clinical trial demonstrated that inhibiting MMPs resulted in an accelerated wound healing, faster recovery and reduced pain in patients with aphthous ulcerations [59]. Therefore, it is not surprising that subjects in our test group reported lower pain scores and painkillers assumption within the first two weeks. Additionally, we employed a novel interactive mobile app for capturing further details of patients' post-operative morbidity measures. This as well could have contributed to the detection of additional differences in PROMs which may have not been noticed with traditional questionnaires. This interactive app was previously validated as a consistent and reliable method for tracking and analyzing pain, showing greater precision than traditional VAS [41].

Relative to our US tissue perfusion analysis, we noted a significant difference between the test and control for at the 2-week time point. Color velocity and color power, which visualize the speed and the amount of blood flowing, respectively, were significantly elevated in the test group compared to the control group in both the midfacial and interproximal areas. In other words, higher tissue perfusion was present in sites that received the growth factor after two weeks. It has been demonstrated that local delivery of rhPDGF has clinical and biological on growth factors release during wound healing and involved in the process of angiogenesis [45]. In particular, rhPDGF was found to increase the amount of vascular endothelial growth factor (VEGF) over the first two weeks, and then gradually decrease to baseline values [45]. While tissue perfusion analysis with power doppler ultrasound has previously been described for soft tissue augmentation at implant sites [26], this is the first report describing its application for evaluating blood flow changes following root coverage procedures, and therefore, comparisons with other studies cannot be performed at the present moment.

Among the limitations of the present study, the relatively short follow-up period must be mentioned. Indeed, a longer follow-up time point would be beneficial to investigate the stability of the obtained outcomes, as well as the fate of the buccal bone. Also, additional ultrasonographic evaluations at earlier time points would have been beneficial for better understanding the effect of rhPDGF on tissue perfusion at the treated sites.

5. Conclusion

Within the limitations of the current study, it was observed that recombinant human platelet-derived growth factor combined with a xenogeneic collagen matrix can enhance the outcomes of root coverage procedures in the treatment of multiple adjacent gingival recession defects with the coronally advanced flap. Greater volume, esthetic and patient-reported outcomes were also observed in the group that received the growth factor.

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Conflict of interest

The authors do not have any financial interests, either directly or indirectly, in the products or information enclosed in this manuscript.

Funding information

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Tables and Figures

Figure 1. Surgical intervention in a patient allocated to the test group (CAF + XCM + rhPDGF). A) Baseline showing multiple adjacent gingival recessions. Only the canine and the two premolars were considered for study measurements. B) Flap design. C) Flap elevation. D) Intraoperative measurement of bone dehiscence. E) Application of 24% EDTA for chemical root conditioning. F) XCM after trimming. G) Sealed envelope containing the randomized solution (rhPDGF in this case). H) XCM saturated with the solution for 15 minutes. I-J) XCM inserted and stabilized below the envelope flap. L) Flap closure. K) 6-month outcomes.

Figure 2. CONSORT Flowchart

Figure 3. Clinical, volumetric and ultrasonographic outcomes of test and control groups

Figure 4. Ultrasonographic tissue perfusion changes over time at the midfacial and interproximal areas

Table 1. Study population and baseline characteristics of the study sites within the two groups

Table 2. Clinical, volumetric and esthetic outcomes at the 3 and 6-month follow-up

Table 3. Ultrasonographic linear outcomes changes over time

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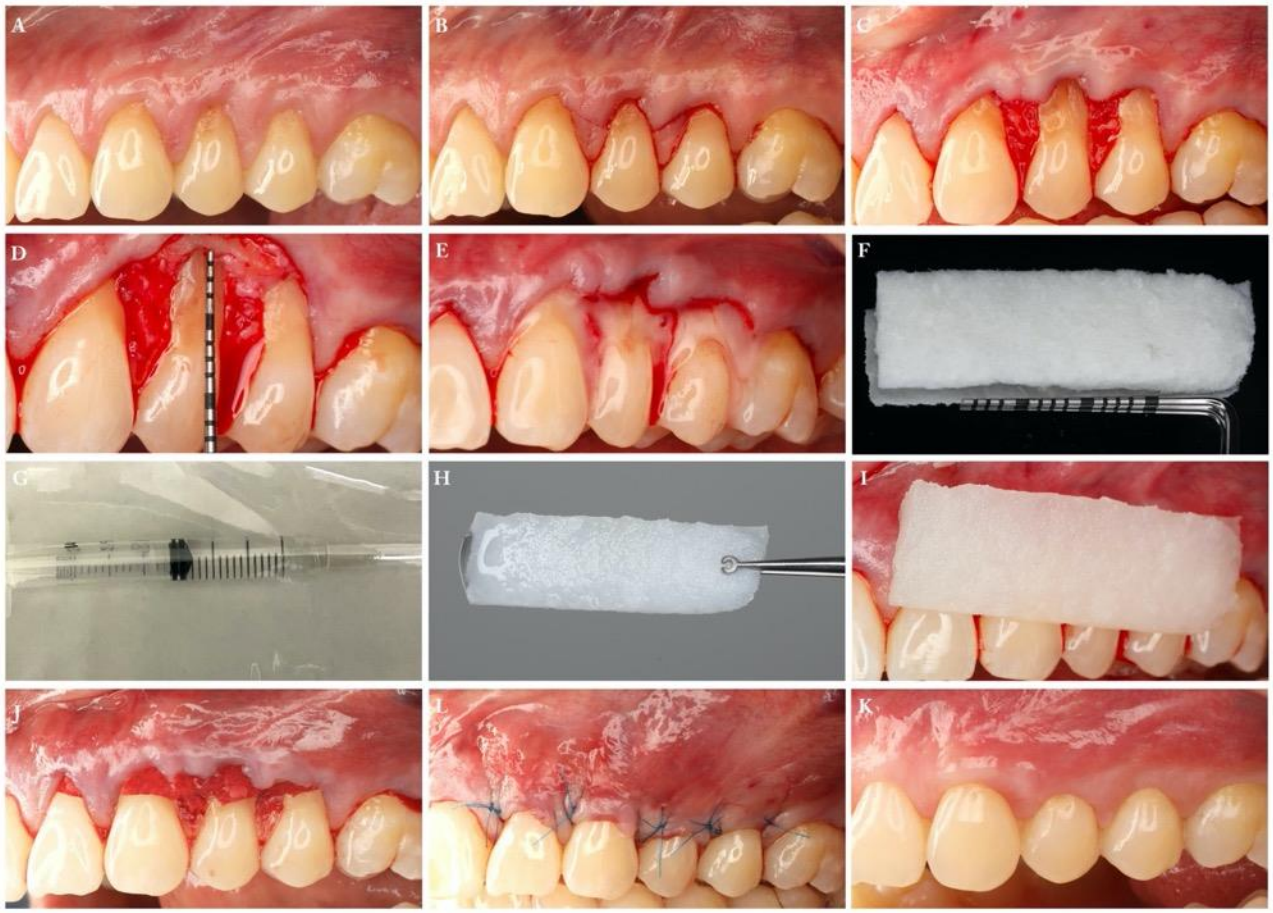

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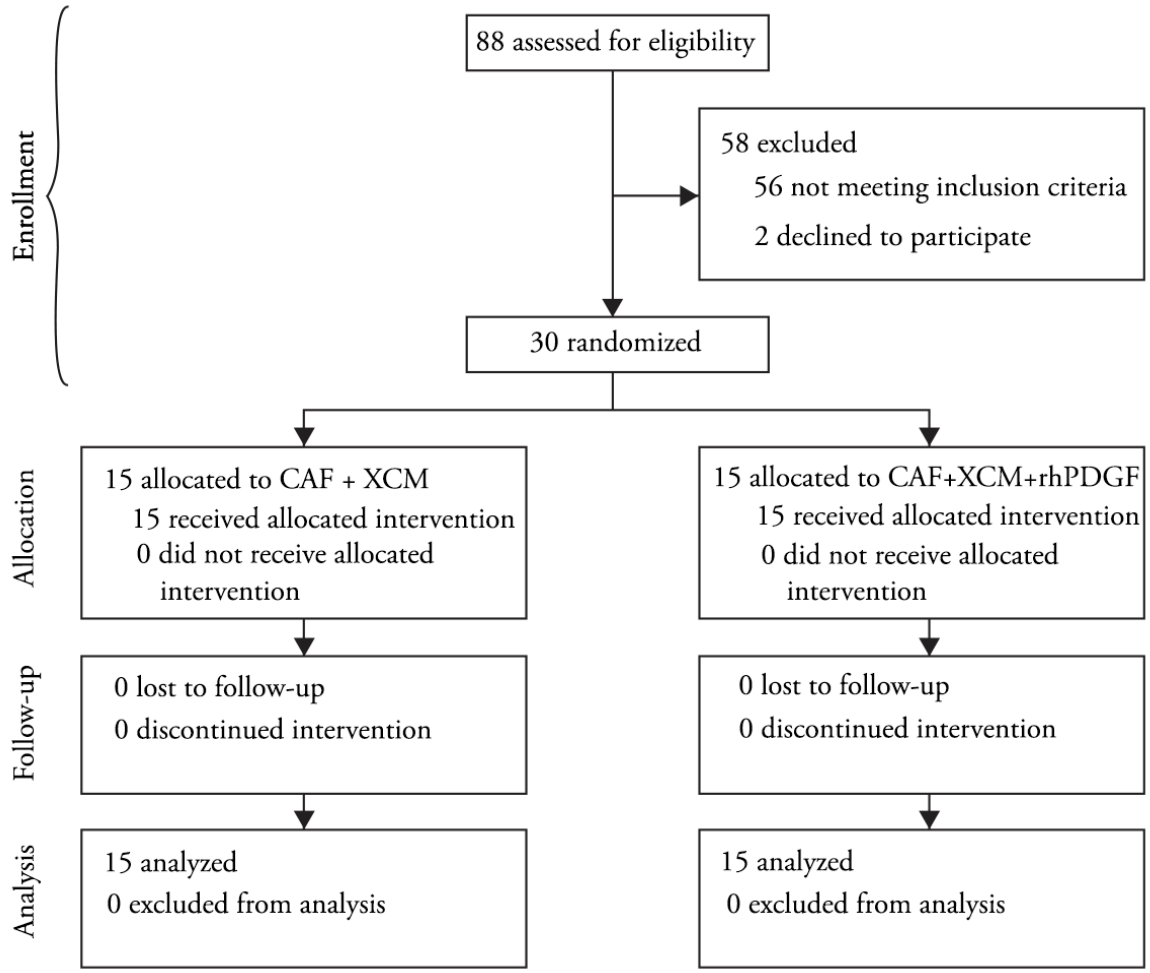
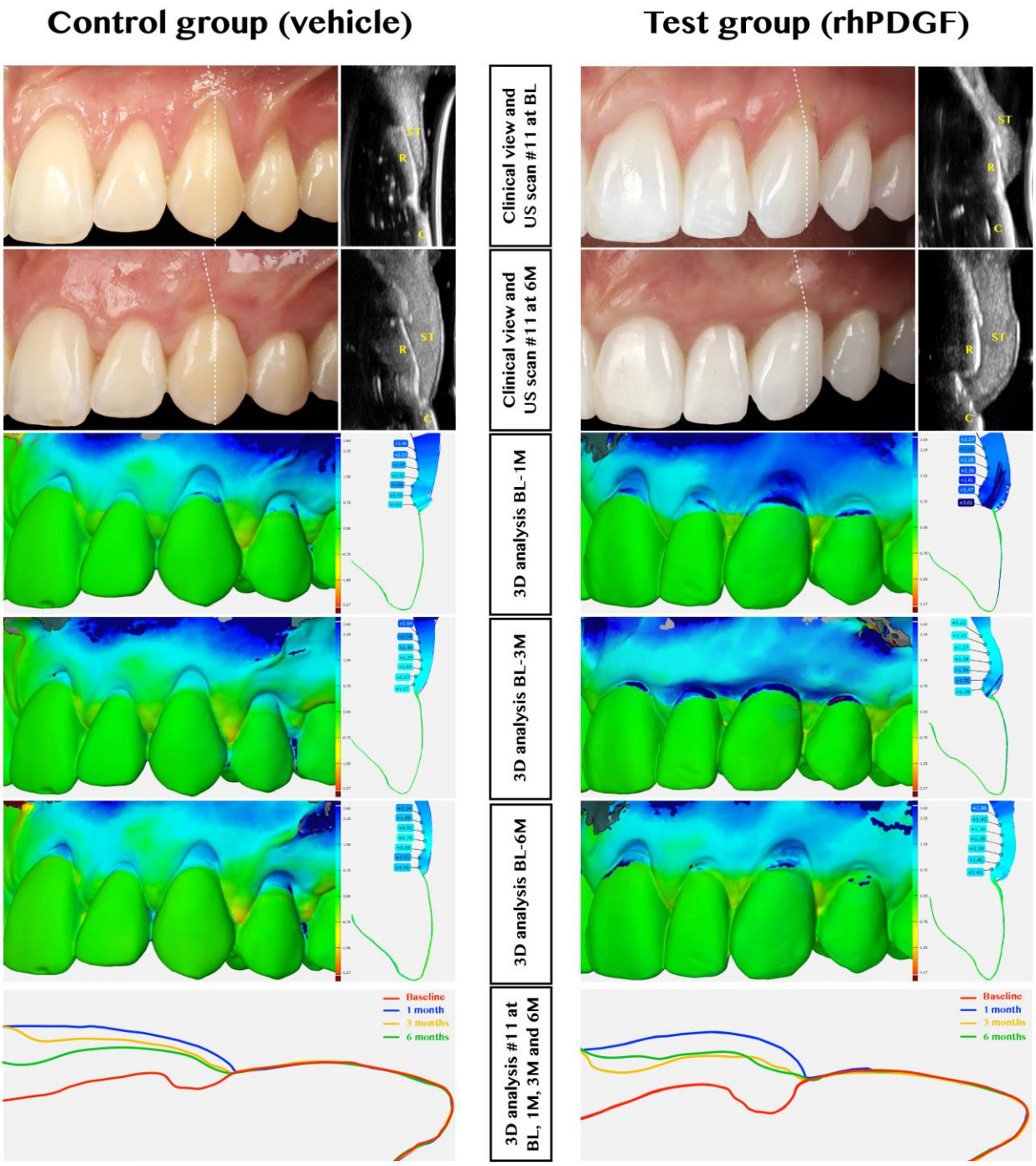
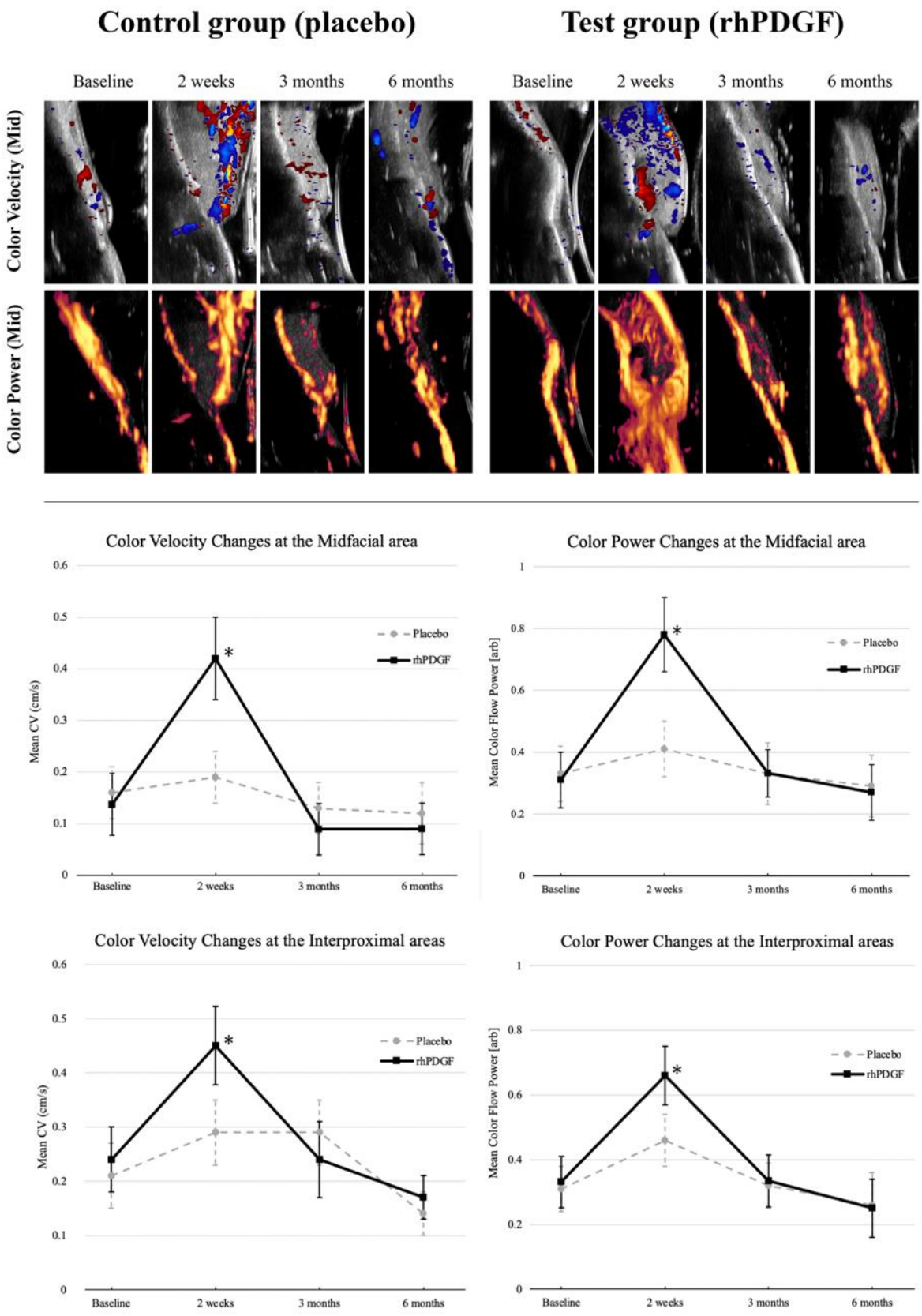


Figure 3. Clinical, volumetric and ultrasonographic (US) outcomes of test and control groups



Legend. BL: baseline. C: crown. R: root. ST: soft tissue. 1M: 1 month. 3M: 3 months. 6M: 6 months.

Figure 4. Ultrasonographic tissue perfusion changes over time at the midfacial and interproximal areas



Legend. Arb. Arbitrary unit. cm/s: centimeter per second. CV: color velocity. Mid: Midfacial aspect of the tooth.

* denotes statistically significantly higher values compared to the other group ($p < 0.01$)

Table 1. Study population and baseline characteristics of the study sites within the two groups

Parameter	Control group (CAF + XCM)	Test group (CAF + XCM + rhPDGF)
Age (mean ± SD) (years)	40.9 ± 12.3	36.0 ± 11.0
Females (N)/(%)	8/53.3	11/73.3
Smokers (N)	1	0
Total Sites (N)	44	47
Maxillary/mandibular sites (N)	31/13	35/12
Sites with NCCLs (N)	7	6
Sites in which the CEJ was reconstructed (N)	7	6
Rec depth (mean ± SD) (mm)	2.97 ± 1.22	2.87 ± 0.79
PD (mean ± SD) (mm)	1.67 ± 0.63	1.37 ± 0.49
CAL (mean ± SD) (mm)	4.64 ± 1.48	4.24 ± 1.10
KTW (mean ± SD) (mm)	2.10 ± 1.28	2.50 ± 0.88
GT (mean ± SD) (mm)	0.84 ± 0.27	0.86 ± 0.26

Legend. CAL: clinical attachment level. GT: gingival thickness. KTW: keratinized tissue width. NCCLs: non-carious cervical lesion. PD: pocket depth. Rec: recession. SD: standard deviation.

Table 2. Clinical, volumetric, and esthetic outcomes at the 3- and 6-month follow-up visits.

Outcome	Control group (CAF + XCM)		Test group (CAF + XCM + rhPDGF)	
	3 months	6 months	3 months	6 months
Clinical outcomes				
mRC (mean ± SD) (%)	82.75 ± 22.79	84.32 ± 21.10	90.62 ± 25.93*	90.50 ± 17.42*
CRC (%)	54.29	54.55	75.61*	71.88*
PD change (mean ± SD) (mm)	0.57 ± 0.67	0.52 ± 0.59	0.01 ± 0.68	0.14 ± 0.50
CAL gain (mean ± SD) (mm)	3.00 ± 1.39	2.50 ± 0.82	2.63 ± 1.30	2.64 ± 0.99
KTW gain (mean ± SD) (mm)	-0.10 ± 1.05	0.09 ± 1.13	-0.01 ± 0.77	0.05 ± 0.68
GT gain (mean ± SD) (mm)	0.66 ± 0.22	0.61 ± 0.32	0.81 ± 0.36*	0.78 ± 0.34*
Sites with soft tissue phenotype modification (%)	94.29	96.74	100	100
3D Digital outcomes				
Vol (mean ± SD) (mm^3)	33.95 ± 13.63	27.35 ± 10.91	41.44 ± 12.55*	37.32 ± 12.21*
ΔD (mean ± SD) (mm)	0.81 ± 27	0.57 ± 0.27	0.93 ± 0.30*	0.75 ± 0.30*
LD1 (mean ± SD) (mm)	0.56 ± 0.45	0.48 ± 0.39	0.70 ± 0.42*	0.53 ± 0.31
LD3 (mean ± SD) (mm)	1.12 ± 0.49	0.68 ± 0.31	1.21 ± 0.45	0.78 ± 0.27*
LD5 (mean ± SD) (mm)	1.47 ± 0.42	0.81 ± 0.49	1.59 ± 0.37	0.95 ± 0.41*
Esthetic outcomes				
RES (mean ± SD) (points)		7.41 ± 2.06		9.08 ± 1.09*

Legend. CAL: clinical attachment level. CRC: complete root coverage. GT: gingival thickness. KTW: keratinized tissue width. LD" linear dimensional changes. mRC: mean root coverage. PD: pocket depth. RES: root coverage esthetic score. SD: standard deviation. Vol: volume changes in mm^3. ΔD: mean distance between the surface/mean thickness of the reconstructed volume in mm.

* denotes a p-value < 0.05 comparing to the other group.

Note that changes/gain or reduction refer to the specific time point versus baseline.

Table 3. Ultrasonographic linear outcomes changes over time.

Ultrasonographic Outcome	Control group (CAF + XCM)			Test group (CAF + XCM + rhPDGF)		
	2 weeks - BL	3 months - BL	6 months - BL	2 weeks - BL	3 months - BL	6 months - BL
STT1 gain (mean ± SD) (mm)	0.78 ± 0.49	0.28 ± 0.36	0.32 ± 0.22	0.87 ± 1.20	0.34 ± 1.01	0.42 ± 0.31
STT3 gain (mean ± SD) (mm)	2.15 ± 0.86	0.63 ± 0.49	0.64 ± 0.60	2.34 ± 0.89	0.79 ± 0.54 *	0.77 ± 0.47 *
STT5 gain (mean ± SD) (mm)	3.23 ± 0.87	0.93 ± 0.55	0.96 ± 0.53	3.44 ± 1.20	1.15 ± 0.58 *	1.06 ± 0.33 *

Legend. BL: baseline. SD: standard deviation. STT1: soft tissue thickness measured 1 mm apical to the gingival margin. STT3: soft tissue thickness measured 3 mm apical to the gingival margin. STT5: soft tissue thickness measured 5 mm apical to the gingival margin.

* denotes a p-value < 0.05 comparing to the other group.

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