rhPDGF improves Root Coverage of a Collagen Matrix for Multiple Adjacent Gingival Recessions: A Triple-blinded, Randomized, Placebo-controlled Trial

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Supplementary Appendix

***In-vitro* release study**

Methods.

Using an 8mm diameter biopsy punch, 50 replicate samples of xenogeneic cross-linked collagen matrices (CCM; Geistlich Fibro-Gide, Geistlich Pharma AG, Wolhusen, Switzerland) were fabricated and placed into a 96-well plate (5 replicates x 10 time points). Each sample was injected with 75 µl of 0.3 mg/mL recombinant human platelet-derived growth factor-BB (rhPDGF; GEM21S, Lynch Biologics, Franklin, USA) and incubated for 10 minutes at room temperature. Then, 125 µl of sterile phosphate-buffered saline (PBS) was added to each sample and then incubated on a plate shaker (150 rpm) at 37 °C. At each time point (15', 30', 1, 2, 6, 12, 24, 48, 72, 96 hrs) 110 µl was removed from each of the 5 replicate wells and saved at -20°C for enzyme-linked immunosorbent assay (ELISA) quantification. For measurement of the PDGF protein released over time, an ELISA (R&D Systems, USA) was performed following the manufacturer's instructions. Each sample was thawed and serially diluted to 1:5000 in PBS before assaying. A negative control (PBS) and a positive control (GEM21S; 1:500) were also included. Optical densities of each well were measured using a microplate reader at a wavelength of 450 nm.

Results of the ELISA

The standard curve of the rhPDGF is depicted in Supplementary Figure 1A. After 15 minutes, the optical density analysis revealed a mean of 570.995 ng/ml release of rhPDGF from the CCM. The mean concentrations of rhPDGF observed after 30 minutes, 1 hour, and 2 hours were 478.146 ng/ml, 377.896 ng/ml and 377.896 ng/ml, respectively. A concentration of rhPDGF of 36.107 ng/ml was observed after 6 hours, while the growth factor was not detected following 12-, 48- and 72-hour time points, respectively. Then, the microplate reader measured 119.995 ng/ml and 103.309 ng/ml rhPDGF release from the CCM after 72 and 96 hours, respectively (Supplementary Figure 1B).

Surgical intervention

After local anesthesia, a split-full-split thickness flap was performed with a mini-blade (Mini Blade #67, Salvin Dental Specialties, Charlotte, USA) and a microsurgical periosteal elevator (Hu-Friedy, Chicago, USA) in a way that the interdental papillae were prepared split thickness, while the soft tissue in the mid-buccal portion was elevated full-thickness in the apical direction until exposing approximately 2 mm of buccal bone. The elevation was then continued split thickness using a 15c blade. The flap was released with a deep and a superficial incision, as previously described (de Sanctis and Zucchelli, 2007), until the flap was not able to reach a position approximately 2 mm coronal to the cemento-enamel junction, without residual tension. The anatomical papillae were de-epithelialized using a mini blade (Mini Blade #67, Salvin Dental Specialties, Charlotte, USA) or microsurgical scissors (Hu-Friedy, Chicago, USA). The root surfaces that were previously exposed to the oral cavity were scaled, planed and detoxified using 24% of EDTA for 2 minutes (Barootchi et al., 2018). For both groups, the xenogeneic cross-linked collagen matrix (CCM) was before extraorally trimmed with a 15c blade, in order to obtain a graft 3-4 mm in thickness and 8 mm in height. The width of the matrix was determined based on the characteristics of the recession defects. The XCM was then saturated with micro-injection of 1.5 cc of the solution contained in the randomization envelope. The graft was left in the dappen dish for 15 minutes as previously recommended (Rubins et al., 2013, Rubins et al., 2014). The solution was also applied on the dried root surfaces before stabilizing the graft. Simple interrupted sutures (6/0 and 7/0 PGA, AD Surgical, Sunnyvale, USA) engaging the graft and the de-epithelialized anatomical papillae were performed to stabilizing the XCM at the recipient bed, approximately 1 mm apical to the CEJ covering the root surface. Further stabilization of the graft was also achieved, if necessary, with additional mattress sutures (6/0 and 7/0 PGA, AD Surgical, Sunnyvale, USA) apical to the XCM, engaging the periosteum. The flap was then coronally advanced and stabilized approximately 2 mm coronal to the cemento-enamel junction using multiple sling sutures at the level of the papillae (6/0 and/or 7/0 polypropylene [Ethicon, Johnson & Johnson, Somerville, USA] or [AD Surgical, Sunnyvale, USA)]), completely covering the XCM. Simple interrupted sutures were performed at the level of the vertical releasing incisions, if any (7/0 polypropylene [Ethicon, Johnson & Johnson, Somerville, USA]).

Post-operative regimen

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.

The final constructed model for the analysis of the primary outcome of mean root coverage at 6 months

The model structure and formula syntax for assessing the efficacy of rhPDGF relative to the primary outcome of mRC at 6 months between the two treatment groups (test vs. control):

$$mRC \sim Treatment + (1|Px\_ID) + (1|Px\_Site)$$

Note that mRC refers to the continuous outcome of percent root coverage per treated site (tooth).
Treatment is an indicator for either groups of test (growth factor) or control (placebo).
Px_ID and Px_Site are random effects for patients, and sites within patient

Supplementary Table 1. Study population and baseline characteristics of the study sites. No statistically significant differences were observed between the two groups at baseline.

Parameter	Matrix + saline	Matrix + rhPDGF
Age (mean ± SD) (years)	40.9 ± 12.3	36.0 ± 11.0
Females (N)/(%)	8/53.3	11/73.3
Smokers (≤ 10 cig/day) (N)	1	0
Total Sites (N)	44	47
Sites with NCCLs (N)	7	6
Sites in which the CEJ was reconstructed (N)	7	6
Rec depth (mean ± SD) (mm)	3.05 ± 1.21	2.87 ± 0.78
PD (mean ± SD) (mm)	1.46 ± 0.61	1.39 ± 0.54
CAL (mean ± SD) (mm)	4.51 ± 1.59	4.27 ± 0.89
KTW (mean ± SD) (mm)	2.10 ± 1.28	2.48 ± 0.87
GT (mean ± SD) (mm)	0.84 ± 0.27	0.92 ± 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.

Supplementary Table 2. Intraoperative measurements of the xenogeneic collagen matrix. No significant differences were observed between the two groups.

Graft dimension	Matrix + saline	Matrix + rhPDGF
Length (mean ± SD) (mm)	28.75 ± 9.22	28.62 ± 6.19
Height (mean ± SD) (mm)	8.21 ± 1.16	8.26 ± 1.52
Thickness (mean ± SD) (mm)	3.52 ± 0.56	3.42 ± 0.46

Legend. SD: standard deviation.

Supplementary Table 3. Esthetic evaluation at the 6-month follow-up using the Root coverage Esthetic Score.

Parameter	Matrix + saline	Matrix + rhPDGF
GM (mean ± SD) (points)	3.61 ± 1.22	4.79 ± 1.49*
MTC (mean ± SD) (points)	0.84 ± 0.37	0.87 ± 0.34
STT (mean ± SD) (points)	0.77 ± 0.42	0.74 ± 0.44
MGJ (mean ± SD) (points)	0.82 ± 0.39	0.85 ± 0.36
GC (mean ± SD) (points)	0.93 ± 0.25	0.91 ± 0.28
Final RES (mean ± SD) (points)	6.98 ± 1.41	8.17 ± 1.99*

Legend. GC: gingival color; GM: level of the gingival margin; MGJ: alignment of the mucogingival junction; MTC: marginal tissue contour; RES: root coverage Esthetic Score; SD: standard deviation; STT: soft tissue texture. * denotes statistical significance based on $p<0.05$ threshold from the mixed-model, in favor of the rhPDGF group.

Supplementary Table 4. Volumetric outcomes from the digital analysis.

Outcome	Matrix + saline		Matrix + rhPDGF	
	BL – 3 months	BL – 6 months	BL – 3 months	BL – 6 months
Vol (mean ± SD) (mm^3)	79.83 ± 44.08	58.67 ± 32.98	96.12 ± 45.97*	75.39 ± 24.76*
ΔD (mean ± SD) (mm)	1.01 ± 0.52	0.73 ± 0.35	1.19 ± 0.91*	0.91 ± 0.19*

Legend. BL: baseline. SD: standard deviation. Vol: volumetric change in mm^3. ΔD: mean thickness of the reconstructed volume. * denotes statistical significance based on $p<0.05$ threshold from the mixed-model, in favor of the rhPDGF group.

Supplementary Table 5. Patient-reported outcome measures (PROMs) at baseline and 6 months.

Outcome	Matrix + saline		Matrix + rhPDGF	
	Baseline	6 months	Baseline	6 months
EST (mean ± SD) (VAS)	31.0 ± 25.1	92.8 ± 8.9	26.5 ± 15.6	88.4 ± 12.0
EST improvement (mean ± SD) (VAS)		61.8 ± 23.7		61.9 ± 19.3
DH (mean ± SD) (VAS)	37.1 ± 26.4	10.3 ± 11.1	35.3 ± 21.5	9.6 ± 15.4
DH reduction (mean ± SD) (VAS)		26.8 ± 24.3		25.8 ± 23.7
SAT (mean ± SD) (VAS)		89.1 ± 12.2		90.0 ± 11.4

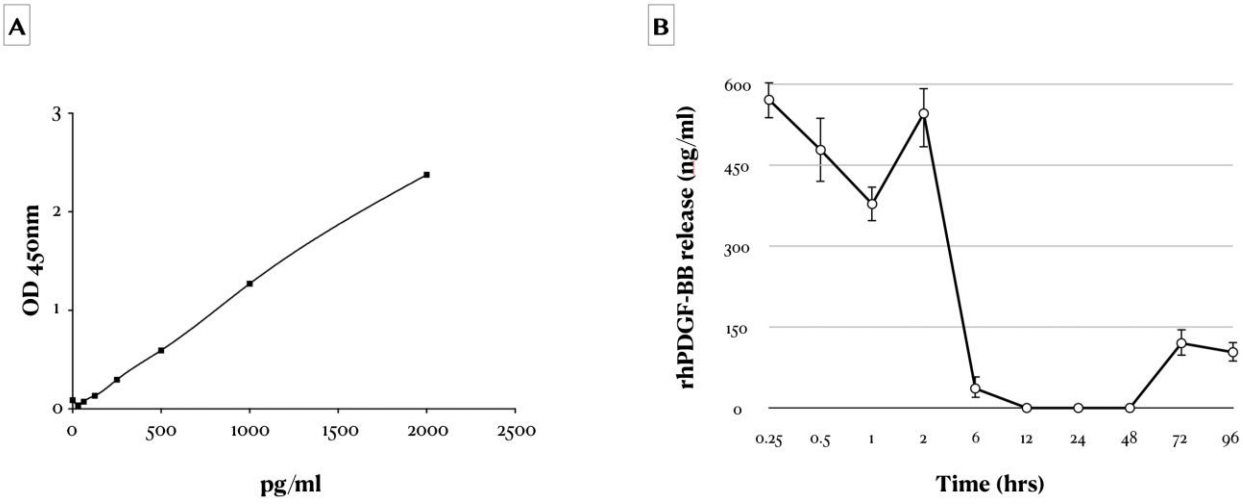
Legend. DH: dental hypersensitivity. EST: esthetic evaluation. SAT: treatment satisfaction. SD: standard deviation. VAS: visual analogue scale.

Supplementary Table 6. Patient-reported dental hypersensitivity (DH) at baseline and 6 months.

Outcome	**Matrix + saline** (15 subjects, 44 sites)		**Matrix + rhPDGF** (15 subjects, 47 sites)	
	Baseline (N, %)	6 months (N, %)	Baseline (N, %)	6 months (N, %)
No DH (VAS = 0)	6, 13.6	11, 25.0	0, 0	16, 34.0
Presence of DH (VAS ≥ 1)	38, 86.4	33, 75.0	47, 100	31, 66.0
DH ≤ 10 VAS	10, 22.7	27, 61.4	5, 10.6	30, 63.8
DH ≥ 50 VAS	15, 34.1	1, 2.3	10, 21.3	1, 2.1

Legend. DH: dental hypersensitivity. N: number of sites. VAS: visual analogue scale.

Supplementary Figure 1. Standard curve (A) and *in vitro* release profile of rhPDGF-BB from xenogeneic cross-linked collagen matrix (B) from the ELISA.



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