Growth Factor–Mediated Treatment of Recession Defects: A Randomized Controlled Trial and Histologic and Microcomputed Tomography Examination

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Background: The primary aims of this two-part prospective study were: 1) to compare the safety and efficacy of beta-tricalcium phosphate (β-TCP) + 0.3 mg/ml recombinant human platelet-derived growth factor-BB (rhPDGF-BB) with a bioabsorbable collagen wound-healing dressing and a coronally advanced flap (CAF) to a subepithelial connective tissue graft (CTG) in combination with a CAF in subjects with gingival recession defects using a randomized, controlled, split-mouth design; and 2) to compare, through histologic and microcomputed tomography (micro-CT) examination, the periodontal regenerative potential of these two therapies in surgically created gingival recession defects in restoring missing cementum, periodontal ligament (PDL), and supporting alveolar bone.

Methods: In the randomized controlled trial (RCT), 30 patients with Miller Class II buccal gingival recession, ≥3 mm deep and ≥3 mm wide in contralateral quadrants of the same jaw were treated and followed for 6 months. Using a split-mouth design with similar bilateral recession defects, test sites were treated with 0.3 mg/ml rhPDGF-BB + β-TCP + bioabsorbable collagen wound-healing dressing; contralateral control sites were treated with a CTG, each in combination with a CAF. In the histologic/micro-CT study segment, recession defects were created in six teeth, each requiring extraction for orthodontic therapy. These defects were created with a recession depth ≥3 mm, the osseous crest 2 to 3 mm apical to the gingival margin, and with 2 to 3 mm of keratinized tissue. The defects were treated with a CTG (control) or rhPDGF-BB + β-TCP + wound-healing dressing (test), plus CAF. Nine months after surgical correction, en bloc resections were obtained and examined histologically and with micro-CT.

Results: In the RCT, test and control treatments demonstrated clinically significant improvements from baseline through month 6. Statistically significant results favoring the CTG were found in recession depth reduction (–2.9 + 0.5 mm, test; –3.3 + 0.6 mm, control; $P = 0.009$), root coverage (90.8%, test; 98.6%, control; $P = 0.013$), and –3.9 ± 0.7 mm, control, –3.3 ± 1.3 mm, test, recession width reduction ($P = 0.035$), whereas mid-buccal probing depth (PD) and PD reduction (PDR) reduction favored the test group (1.4 ± 0.4 mm, test; 1.8 ± 0.1 mm, control; $P < 0.001$ PD and –0.0 mm test; +0.4 mm control PDR). For all other parameters, the two treatments were statistically equivalent, including increases in keratinized tissue, esthetic results, and subject satisfaction. In the histologic/micro-CT portion, all four sites treated with rhPDGF-BB + β-TCP showed evidence of regeneration of cementum, PDL with inserting connective tissue fibers, and supporting alveolar bone, whereas neither CTG-treated site exhibited any signs of periodontal regeneration.

Conclusions: CTG and rhPDGF-BB + β-TCP + wound-healing dressing are effective treatment modalities for clinically correcting gingival recession defects. In addition, the current study demonstrated that regeneration of the periodontium in gingival recession defects was possible through a growth factor–mediated approach. *J Periodontol 2009;80:550-564.*

KEY WORDS

Connective tissue; gingival recession/surgery; grafts; histology; recombinant platelet-derived growth factor-BB; rhPDGF-BB.

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doi: 10.1902/jop.2009.080502

The successful resolution of patient-centered concerns related to gingival recession defects, i.e., improved esthetics, reduction or elimination of root dentinal sensitivity, decreased incidence of cervical caries, and improved ability to control plaque is critical to effective treatment outcomes when addressing recession-related mucogingival deficits. An additional, although perhaps more elusive, therapeutic goal is the regeneration of the lost attachment apparatus common to all recession defects, including the formation of new cementum with inserting connective tissue fibers and supporting alveolar bone.[1] Two recent systematic reviews[2,3] of randomized controlled clinical trials verified the effectiveness of the subepithelial connective tissue graft (CTG), guided tissue regeneration (GTR), and the coronally advanced flap (CAF) in producing statistically significant gains in root coverage and clinical attachment. In both reviews, the subepithelial CTG seemed to be more effective than GTR in resolving or reducing gingival recession. Additional studies[4,5] examining the long-term results of the CTG seem to support its long-term efficacy in maintaining root coverage. Supported by such evidence-based data, the CTG is viewed by many clinicians as the gold standard treatment in reducing or eliminating gingival recession. Less certain, however, is its ability to satisfy the other major goal of recession therapy: regeneration of the lost attachment apparatus, including the formation of new cementum with inserting connective tissue fibers and supporting alveolar bone.

A number of studies suggested that the CTG has at least limited potential to regenerate tissues of the periodontium. In a case study examining a tooth removed en bloc following root fracture, Harris[6] demonstrated regeneration coronal to the original gingival margin. Likewise, Goldstein et al.[7] demonstrated regeneration of new bone, cementum, and periodontal ligament (PDL) 15 months after CTG with a CAF in a maxillary first bicuspid with 5 mm of recession. Bruno and Bowers[8] also found 0.5 to 1 mm of regenerated bone, cementum, and PDL just coronal to the alveolar crest, with connective tissue covering the majority of the remaining exposed root surface.

Although some evidence for regeneration exists, other studies suggested that attachment through a long junctional epithelium (LJE) or connective tissue adhesion is more likely to result following connective tissue grafting. In a study examining shallow recessions using a double-pedicled graft with connective tissue, Harris[9] demonstrated an LJE extending close to the original bony crest or connective tissue fibers running parallel to the root surface. In a histologic study examining en bloc resections of maxillary premolars following connective tissue grafting, Majzoub et al.[10] also found predominantly an LJE adjacent to exposed dentin along with evidence of parallel-oriented connective tissue fibers. In a third histologic study, Cummings et al.[11] compared the CTG to acellular dermal matrix and reported findings for each to be a combination of an LJE and connective tissue, without evidence of periodontal regeneration.

Although considered by many as the most effective treatment for root coverage, the CTG has a number of disadvantages: a distant donor site is required, increased morbidity may be associated with graft harvest, and limited amounts of donor tissue may increase the total treatment time for patients with multiple recession defects. Considering these limitations, as well as the variable results seen in regenerating tissues of the attachment apparatus, recombinant growth factor technology may offer viable alternatives to the CTG, including regeneration of cementum, PDL, and supporting alveolar bone.

Since the late 1980s, when research first revealed that platelet-derived growth factor (PDGF) promoted regeneration of cementum, inserting connective tissue fibers and bone, >100 studies have underscored PDGF's positive effects on chemotaxis and the proliferation of animal and human PDL and alveolar bone cells.[12-14] Several recent human histologic studies[15-17] dramatically revealed PDGF's ability to support periodontal regeneration in severe periodontal interproximal and Class II furcation defects.

Based on the positive clinical and histologic findings of the preceding studies, McGuire and Scheyer,[18] in a split-mouth case series, recently evaluated multiple clinical parameters following the treatment of gingival recession defects with beta-tricalcium phosphate (β-TCP) and a collagen membrane, both saturated with recombinant human PDGF-BB (rhPDGF-BB). Comparing the results achieved after 6 months with PDGF to those achieved with the subepithelial CTG, both procedures achieved root coverage with ≤1 mm of residual recession depth. Similar improvements were seen in probing attachment levels and height of keratinized tissue for the experimental treatment group and the CTG group. Given the favorable tissue responses to rhPDGF-BB with β-TCP and a collagen membrane, and results comparable to the CTG, a properly powered, controlled clinical trial was deemed warranted.

Based upon the above initial feasibility study findings as well as earlier histologic regenerative findings in restoring the complete attachment apparatus with an rhPDGF-BB–mediated approach for intrabony and Class II furcation defects, a single-center, two-part prospective study was undertaken with the following objectives: 1) to compare, in subjects with recession-type defects, the safety and efficacy of β-TCP + 0.3 mg/ml rhPDGF-BB with a bioabsorbable collagen wound-healing dressing‡ and a CAF to a subepithelial CTG in combination with a CAF using a randomized,

‡ CollaTape, Integra LifeSciences, Carlsbad, CA.

controlled, split-mouth design; and 2) to compare the periodontal regenerative potential of the above two therapies in surgically created gingival recession defects in restoring missing cementum, PDL, and supporting alveolar bone through histologic and micro-computed tomography (micro-CT) examinations.

MATERIALS AND METHODS

Part I: Randomized Controlled Trial (RCT)

Study population. Thirty patients (four males and 26 females) with Miller Class II[19] buccal gingival recession ≥3 mm deep and ≥3 mm wide in contralateral quadrants of the same jaw who met the inclusion/exclusion criteria were eligible to enter the study. Inclusion criteria included the following: patients must have read, understood, and signed an informed consent form; been able and willing to follow study procedures and instructions; had at least one pair of teeth with buccal recession type defects ≥3 mm deep and ≥3 mm wide (selected defects were located in contralateral quadrants of the same jaw, with similar size and morphology with respect to depth and width, and selected teeth subjected to root canal treatment were asymptomatic and without technical remarks; the root canal treatment should have been completed at least 6 months before inclusion); had presence of keratinized tissue; and in case of adjacent teeth with recession defects, only one tooth at each side acted as a test or control tooth; no adjacent teeth were grafted during surgeries. Exclusion criteria included the following: subjects who were participating in other clinical trials; had a history of cancer or human immunodeficiency virus; had an allergy to yeast- derived products; failed to maintain good plaque control; had Class V restorations; had teeth with extremely prominent root surfaces; had any systemic acute infections in the areas intended for surgery; had a history within the previous 6 months of weekly or more frequent use of smokeless chewing tobacco, pipe or cigar smoking and cigarette smoking; had taken chronic therapeutic doses of medication known to affect bone metabolism; and female subjects who were pregnant or lactating or sexually active females who were of childbearing potential and were not using hormonal or barrier methods of birth control. The following teeth did not qualify: molars; teeth with axial mobility; teeth with interproximal loss of attachment; and teeth with crowns or veneers. All patients were enrolled from the authors' (MKM and ETS) private practice from June to October 2006. The patient population included subjects 18 to 70 years of age (mean age: 43.8 ± 10.7 years). A signed institutional review board–approved consent form was obtained from each patient. Of the 30 patients, one was of Hispanic origin, and the rest were white. All of the patients were current non-smokers; 14 had smoked 11.0 ± 11.4 cigarettes/day for a mean of 6.6 ± 6.6 years. No patient smoked in the 6 months prior to study entry. All treated defects required marginal gingival keratinized tissue. At baseline, keratinized tissue measured 1.9 ± 0.6 mm for the rhPDGF-BB group and 1.9 ± 0.8 mm for the subepithelial CTG group. All occlusal interferences were removed through occlusal adjustment, and biteguards were constructed as needed. The split-mouth design of this study allowed each patient to serve as his or her own control.

Clinical evaluation. At baseline and at each post-surgical follow-up visit, the treated sites were examined clinically, photographic documentation was performed, and defect measurements were recorded. Following initial baseline screening and surgery, six follow-up visits through week 24 were mandated for this study. Radiographs were taken at baseline and at week 24. The primary efficacy endpoint was the change in recession depth at 6 months. Secondary endpoints included clinical attachment level (CAL), probing depth (PD), PD reduction (PDR), height of keratinized tissue, percentage of root coverage, recession width, clinician rating of color/texture of treatment sites, the subject's esthetic satisfaction, and subject perception of pain/discomfort.

Baseline parameters included the following: gingival recession depth, recession width measured at the level of the cemento-enamel junction (CEJ), height of keratinized tissue from the free gingival margin to the mucogingival margin, buccal and proximal PDs, CAL measured from the CEJ, alveolar bone level, root dentin hypersensitivity, inflammation score, and plaque score for both test teeth. At baseline, there were no statistical differences between the teeth allocated to the two study treatments with regard to recession depth ($P = 0.349$), CAL ($P = 0.672$), mid-buccal PD ($P = 0.681$), proximal PD of the mesial surface ($P = 0.464$), proximal PD of the distal surface ($P = 0.804$), height of keratinized tissue ($P = 1.000$), or recession width ($P = 0.922$).

Evaluation of healing, based on the presence or absence of inflammation using a visual analog scale, with "much worse than expected" on one end and "much better than expected" on the other end, was made at weeks 1, 2, and 4.

Root dentin hypersensitivity was assessed using a conventional blast of air for 3 seconds at the exposed root surface. The hypersensitivity was recorded as none, mild, moderate, or severe.

Visible signs of inflammation were examined and recorded based on the modified gingival index of Lamster et al.[20]

Plaque scores of test and control teeth were calculated based on the presence (1) or absence (2) of plaque on the buccal and lingual surfaces of the study teeth.

Gingival color and texture were assessed by comparing test and control teeth to surrounding tissues and

scoring through questionnaires, i.e., more red, less red, or equally red and more firm, less firm, or equally firm.

The patient's perception of pain was assessed using a visual analog scale, with "no pain" and "extreme pain" on each end, for graft and donor sites.

A visual analog scale measuring the patient's esthetic satisfaction, with "disappointed" at one end and "fully satisfied" at the other end, was completed at baseline and week 24.

All pre- and postoperative clinical assessments were made by a single, masked examiner who was not the operating surgeon. Training and calibration were conducted prior to study initiation to ensure intraexaminer reproducibility of measured outcomes.

Surgical procedure. All subjects received oral hygiene instructions and were not scheduled for surgery until they were capable of demonstrating adequate supragingival plaque control. Immediately prior to surgery, the treating surgeon (or delegated dental assistant) opened an envelope with the subject's identifying number and treatment assignment. The subepithelial CTG and rhPDGF-BB + β-TCP treatments were assigned to the right or left side of the subject's mouth according to a predetermined randomization schedule. Subjects were not informed about which treatment each study tooth had received. The examining clinician remained masked to the randomization code for the duration of the study.

Test and control sites were treated as described recently by McGuire and Scheyer,[18] with the following exception: a bioabsorbable collagen wound-healing dressing§ saturated with rhPDGF-BB was placed over the grafted test root surfaces in place of a collagen barrier membrane. The first surgery was performed on the left side in all subjects. The second surgery was performed immediately after the first surgery.

Statistical methods. Descriptive statistics were performed by treatment and provided the summaries for continuous variables displayed as sample size (n) and mean and its SE, SD, median, and range and for categorical variables displayed as count and percentage.

One-way analysis of variance (ANOVA) was used to compare the difference between treatments for the continuous measurements. The Fisher exact test was used to compare the difference between treatments for the categoric parameters.

For exploratory purposes, analysis of covariance (ANCOVA) was also used for the primary and secondary variables (CAL, PDR, height of the keratinized tissue, and recession width); the treatment comparison was adjusted for the baseline value, site (left/right), and sequence of treatments.

All statistical tests were two-sided.

Sample size determination. Power calculations at the 5% significance level determined that 20 evaluable subjects were needed to detect a 1.0-mm change in recession depth, with 95% power and assuming a within-subject variation (SD, estimated from previous studies with similar inclusion/exclusion criteria) of 1.0 mm. This study elected to recruit 30 subjects with buccal recession–type defects. All 30 subjects completed the study.

Part II: Histologic/Micro-CT Examination

Study population. Two patients were enrolled in this portion of the study. Each required first premolar extraction for orthodontic treatment. One patient required the removal of all four first premolars, whereas only the maxillary first premolars required extraction in the second patient. Therefore, six teeth were scheduled for removal. Gingival recession defects were surgically created buccal to all six of these teeth. Following a thorough explanation of the nature of this portion of the study, which included information regarding the potential risks as well as an understanding of the lack of any clinical benefit derived from recession-related procedures, informed consents were signed, and institutional review board approval was obtained. For participating in this study, orthodontic treatment was provided without cost to the patients who otherwise could not afford orthodontic care.

Basic study design. Surgically created recession defects were designed with the following characteristics: recession depth ≥3 mm, osseous crest 2 to 3 mm apical to the apically positioned gingival margin, and a maximum of 2 to 3 mm of keratinized marginal gingiva (Fig. 1). In an effort to replicate a naturally contaminated root surface, the surgically created defects were left exposed to the oral environment for 2 months. Four of the six defects were grafted with rhPDGF-BB + β-TCP + a collagen wound-healing dressing, and two were grafted with CTGs. As in the RCT portion of the study, CAFs were used to cover the grafted sites (Figs. 2 and 3). Discrete reference notches were placed at the pregrafted free gingival margin and at the osseous crest of all sites (Fig. 4). In each patient, one tooth, as determined by a randomization schedule, served as the control and received a CTG. All other premolars received the rhPDGF-BB–mediated therapy. Nine months after correction of the surgically created recession defects, conservative en bloc resections were obtained for histologic and micro-CT examination. The technique used preserved as much ridge as possible to minimize the chance for a ridge defect following the en bloc resection. The following technique required precise incisions into soft tissue, followed by cuts into hard tissue. A full-thickness incision was made from each of the line angles of the buccal free gingival margin to the center of the adjacent papillae. Then a vertical incision was made interproximally through the

§ CollaTape, Integra LifeSciences.

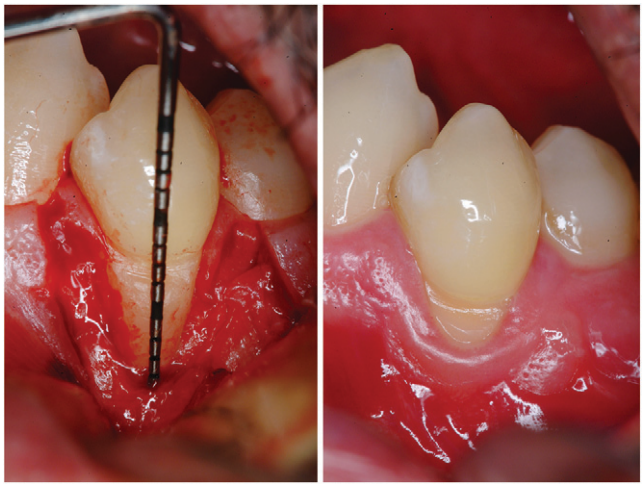

Figure 1.
Surgically created gingival recession defect with osseous crest 2 to 3 mm apical to the newly created gingival margin.

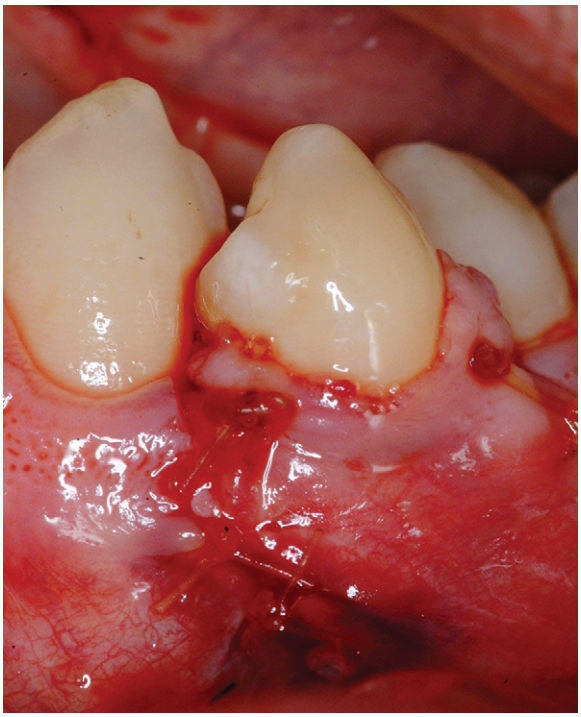

Figure 2.
Gingival recession corrected 2 months after defect creation with rhPDGF-BB + β-TCP or CTG, each with a CAF.

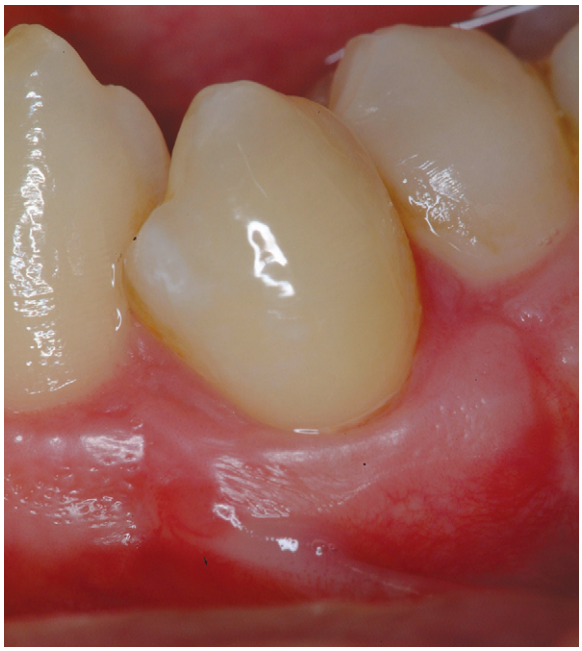

Figure 3.
Nine months after correction of a surgically created recession defect.

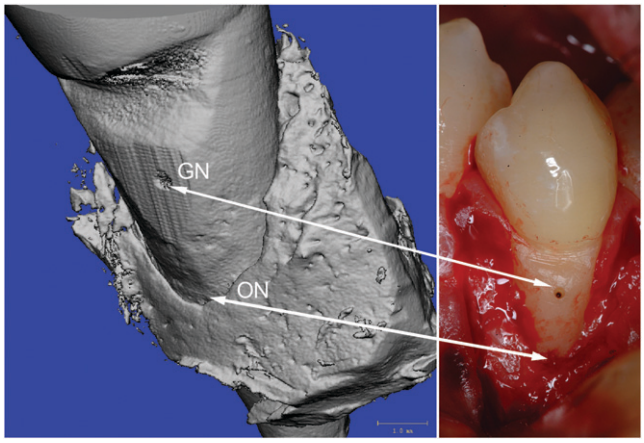

Figure 4.
Reference notches are seen at the preoperative gingival margin and alveolar crest in the surgically created defects prior to correction of the recession defect with rhPDGF-BB + β-TCP or a CTG with CAF. GN = gingival notch; ON = osseous notch.

gingiva, extending into the mucosa. Next, the mesial and distal incisions were connected with an incision parallel to and below the mucogingival junction. The surgeon, using a piezosurgical instrument, cut the tooth from the facial to the lingual aspects, breaking the mesial and distal contacts. As soon as the contact was broken, the cut was angled through the tooth toward the central fossa, with the mesial and distal cuts joining at that point. These cuts were made approximately to the depth of the CEJ. A separate cut was made through the cortical plate and the buccal portion of the root 2 to 3 mm below the pregrafted osseous crest following the full-thickness soft tissue incisions. This cut was connected to the initial incisions with an oblique cut, and the buccal portion of the tooth, including the graft, was removed for analysis.

The remaining part of the tooth was extracted in a routine fashion. The resulting alveolar defects were immediately grafted with freeze-dried bone allograft + rhPDGF-BB. Three months later, active orthodontic therapy recommenced uneventfully. No postoperative complications were observed in any of the test or control sites.

Micro-CT analysis. The specimens were scanned using a high-resolution micro-CT system‖ in multislice mode. Each image data set consisted of ~800 micro-CT slice images.

The specimens were scanned in high-resolution mode with an x-, y-, and z-resolution of 16 μm. The image data sets were used to produce three-dimensional (3D) views of the specimens using a special software.¶

The following parameters were examined using micro-CT analysis: two-dimensional and 3D anatomy of preexisting bone and newly formed bone adjacent to the root surfaces, the 3D configuration of the PDL space, and micro-CT corroboration of histologic findings of periodontal regeneration by identifying new bone connected by a PDL-like space in relation to the gingival margin and osseous reference notches.

Operative procedure for surgically created recession defects. Following the administration of local anesthetic, gingivectomies were performed where required, resulting in 2 to 3 mm of keratinized marginal tissue adjacent to each test and control tooth. To allow elevation of a full-thickness mucoperiosteal flap, mesial and distal vertical releasing incisions were connected to a carefully placed intrasulcular incision. Next, where necessary, ostectomies to remove the buccal cortical plate were performed, enabling the osseous crest to be repositioned 2 to 3 mm apical to the newly positioned gingival margin (Fig. 1). Then the mucoperiosteal flap was apically repositioned, resulting in a recession defect ≥3 mm as required by the study design. 5.0 gut sutures were used to close all incisions (Fig. 5).

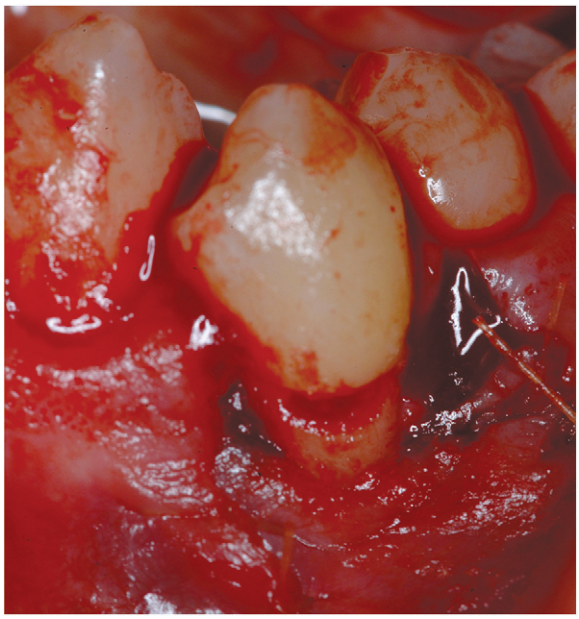

Figure 5.
Surgically created defect with flap apically repositioned to create a recession defect ≥3 mm.

RESULTS

Part I: RCT

Test and control treatments proved to be equally effective and were comparable in terms of patient satisfaction with postoperative discomfort, healing, and the final esthetic result (Fig. 6). On a 10-cm visual analog scale, no statistically significant differences were observed between the treatments in response to an esthetic satisfaction questionnaire. The subjects rated postoperative discomfort, which included bleeding, swelling, and sensitivity, as similar for the two treatment sites. All subjects had mild or no discomfort due to bleeding, swelling, and sensitivity and continued to improve from weeks 1 through 4. No statistically significant difference in pain scores was observed between the study treatments using a 10-cm visual analog scale. At the 24-week postoperative visit, 97% of the subjects commented that they experienced no difference in discomfort between the two treatment sites. Likewise, no statistically significant differences in the clinical rating of color/texture of the tissues were observed between the treatments throughout the study. At week 24, 100% of treatment sites were rated by the masked examiner as equally firm compared to surrounding tissues. One hundred percent of sites treated with rhPDGF-BB were scored as equally red, whereas 90% of CTG sites were rated as equally red compared to surrounding tissues.

Tables 1 and 2 summarize the primary and secondary efficacy endpoints. Test and control treatments resulted in clinically significant improvements from baseline at the 24-week postoperative visit for all measured parameters. For the primary endpoint, change in recession depth at 6 months, treatment with rhPDGF-BB + β-TCP resulted in a mean recession depth reduction of –2.9 ± 0.5 mm, whereas CTG treatment caused a mean reduction of –3.3 ± 0.6 mm (ANOVA, $P = 0.009$; ANCOVA, $P = 0.007$); this statistically significant difference favored the CTG-treated sites. Likewise, although both treatments resulted in significant clinical improvements from baseline, the reduction in recession width and the percentage of root coverage statistically favored the CTG. At 24 weeks, a mean reduction in recession width of

‖ μCT 40, Scanco Medical, Bassersdorf, Switzerland.
¶ Scanco Medical.

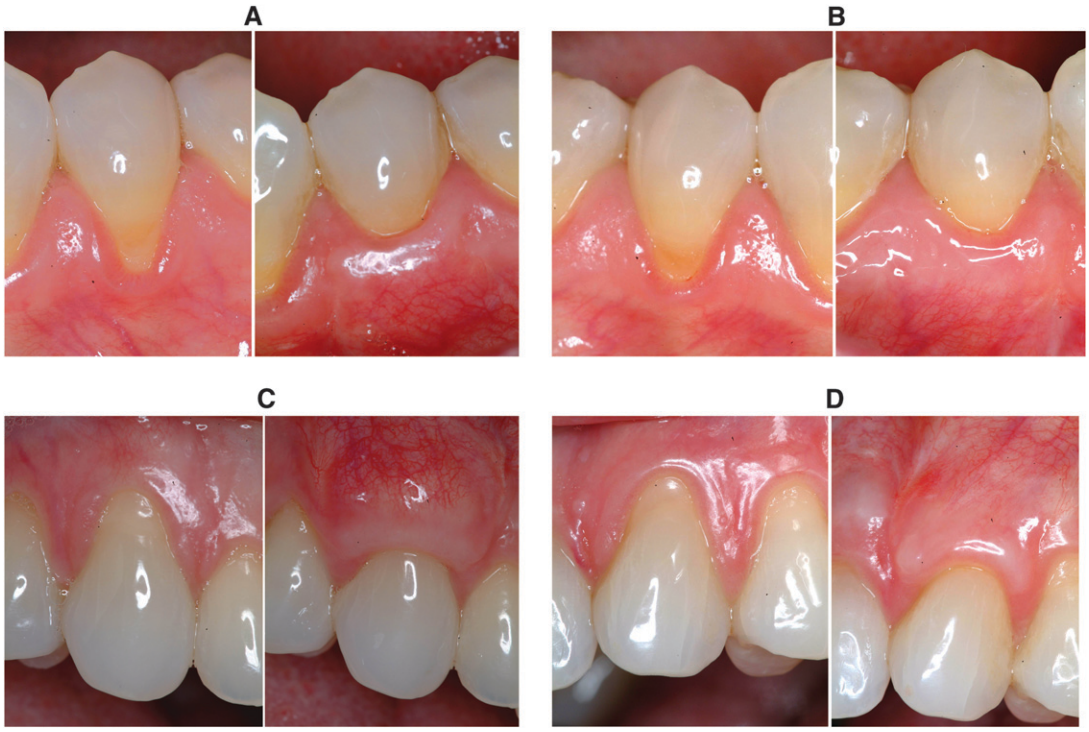

Figure 6.
*Representative control **(A** and **C)** and test **(B** and **D)** pre- (left panel) and postoperative (right panel) sites at baseline and 6 months later.*

–3.3 ± 1.3 mm and –3.9 ± 0.7 mm was seen for the test and control groups, respectively (ANOVA, $P = 0.035$; ANCOVA, $P = 0.021$). The mean percentage of root coverage at 24 weeks was 90.8% and 98.6% for the test and control groups, respectively (ANOVA, $P = 0.013$).

As noted in Table 1, there were no statistically significant differences in the height of keratinized tissue at the 24-week postoperative visit between the study groups' treatments (rhPDGF-BB + β-TCP, 2.9 ± 0.6 mm; CTG, 3.2 ± 0.8 mm; $P = 0.113$). In addition, both treatments resulted in statistically equivalent absolute changes in keratinized tissue from baseline to week 24 (ANOVA, $P = 0.115$; ANCOVA, $P = 0.072$).

Table 2 summarizes the changes in CAL and PD reductions from baseline to week 24 in the test and control groups. CAL was measured, to the nearest 0.5 mm, from the CEJ, the fixed reference point. There were no statistically significant differences in CAL observed between the study treatments at the 24-week postoperative visit (rhPDGF-BB + β-TCP, 1.7 ± 0.5 mm; CTG, 1.8 ± 0.4 mm; $P = 0.335$) or for the absolute change from baseline to the 24-week postoperative visit (rhPDGF-BB + β-TCP, –2.9 ± 0.6 mm; CTG, –2.9 ± 1.0 mm; ANOVA, $P = 0.871$; ANCOVA, $P = 0.385$). PD was measured to the nearest 0.5 mm from the gingival margin to the bottom of the probable pocket of the buccal, mesial, or distal surface. There were no statistically significant differences observed between the study treatments for proximal PD of the mesial or distal surface at the 24-week postoperative visit or for PDR from baseline to the 24-week postoperative visit. However, there was a statistically significant difference between the study treatments for PD of the mid-buccal surface at the 24-week postoperative visit, favoring the test treatment (rhPDGF-BB + β-TCP, 1.4 ± 0.4 mm; CTG, 1.8 ± 0.1 mm; $P < 0.001$) and for the PDR from baseline to the 24-week postoperative visit (rhPDGF-BB + β-TCP, 0.0 ± 0.6 mm; CTG, 0.4 ± 0.6 mm; ANOVA, $P = 0.004$; ANCOVA, $P < 0.001$).

With regard to the safety results, 25 (78.1%) subjects experienced 75 adverse events (AEs) during the study. All AEs were mild or moderate in severity. The most common AE was mild contusion, occurring in 16 (50.0%) subjects, followed by face swelling in 13 (40.6%) subjects. There were no serious AEs during the study.

Part II: Histologic/Micro-CT Examination

Nine months after treatment with a CTG or rhPDGF-BB + β-TCP + a bioabsorbable collagen wound-healing dressing, all six sites retained 100% root coverage. Both therapeutic approaches restored the protective soft tissue morphology and esthetic balance of the mucogingival complex. However, clear and distinct

Table 1.

Recession Depth, Recession Width, Percentage of Root Coverage, and Height of Keratinized Tissue at Baseline and Week 24

Parameter	Baseline	Week 24	Absolute Change From Baseline to Week 24
Recession depth (mm)			
Test			
Mean (SE)	3.2 (0.1)	0.3 (0.1)	−2.9 (0.1)
Range	3 to 5	0 to 2	−4 to −2
Control			
Mean (SE)	3.4 (0.1)	0.1 (0.0)	−3.3 (0.1)
Range	3 to 5	0 to 1	−5 to −3
P value	0.349*	0.015*	0.009*; 0.007†
Recession width (mm)			
Test			
Mean (SE)	4.0 (0.1)	0.7 (0.2)	−3.3 (0.2)
Range	3 to 6	0 to 3	−6 to 0
Control			
Mean (SE)	4.0 (0.1)	0.1 (0.1)	−3.9 (0.1)
Range	3 to 6	0 to 2	−6 to −2
P value	0.922*	0.021*	0.035*; 0.021†
Root coverage (%)			
Test			
Mean (SE)		90.8 (2.8)	
Range		57 to 100	
Control			
Mean (SE)		98.6 (1.0)	
Range		75 to 100	
P value		0.013*	
Height of keratinized tissue (mm)			
Test			
Mean (SE)	1.9 (0.1)	2.9 (0.1)	1.0 (0.1)
Range	1 to 3	2 to 5	0 to 3
Control			
Mean (SE)	1.9 (0.1)	3.2 (0.1)	1.3 (0.1)
Range	1 to 4	2 to 5	0 to 3
P value	1.000*	0.113*	0.115*; 0.072†

* One-way ANOVA.
† ANCOVA model adjusted for recession depth, recession width, and height of keratinized tissue at baseline, side (left/right), and sequence of treatments.

Table 2.

CAL, Mid-Buccal Depth Reduction, and Proximal PDs at Baseline and Week 24

Parameter	Baseline	Week 24	Absolute Change From Baseline to Week 24
CAL (mm)			
Test			
Mean	4.6 (0.1)	1.7 (0.1)	−2.9 (0.1)
Range	4 to 7	1 to 3	−4 to −2
Control			
Mean	4.7 (0.2)	1.8 (0.1)	−2.9 (0.2)
Range	4 to 7	1 to 3	−6 to −2
P value	0.672*	0.335*	0.871*; 0.385†
Mid-buccal depth reduction (mm)			
Test			
Mean	1.4 (0.1)	1.4 (0.1)	0.0 (0.1)
Range	1 to 2	1 to 2	−1 to 1
Control			
Mean	1.4 (0.1)	1.8 (0.1)	0.4 (0.1)
Range	1 to 2	1 to 3	−1 to 1
P value	0.681*	<0.001*	0.004*; <0.001†
Proximal PD (mesial; mm)			
Test			
Mean	2.3 (0.1)	1.9 (0.1)	−0.4 (0.1)
Range	1 to 3	1 to 3	−1 to 1
Control			
Mean	2.2 (0.1)	1.9(0.1)	−0.3 (0.1)
Range	1 to 3	1 to 3	−1 to 1
P value	0.464*	0.871*	0.568*; 0.962†
Proximal PD (distal; mm)			
Test			
Mean	2.3 (0.1)	1.9 (0.1)	−0.4 (0.1)
Range	1 to 3	1 to 3	−2 to 1
Control			
Mean	2.4 (0.1)	2.0 (0.0)	−0.4 (0.1)
Range	2 to 3	2 to 3	−2 to 0
P value	0.804*	0.190*	0.720*; 0.211†

* One-way ANOVA.
† ANCOVA model adjusted for CAL, mid-buccal depth reduction, and mesial/distal proximal PD at baseline, side (left/right), and sequence of treatments.

differences were found in each treatment's ability to restore the lost attachment apparatus common to all recession defects. The following sections examine, in detail, two representative sites from the study (the other sites will be the focus of a companion article): one grafted with a CTG and the other with rhPDGF-BB + β-TCP + a collagen wound-healing dressing, each with a CAF. Both grafted areas are from a well-developed, well-nourished 41-year-old Hispanic male with bimaxillary protrusion associated with severe maxillary and moderate mandibular incisor crowding. The patient required removal of all four first premolars. One site, tooth #21, was grafted with a CTG. The other three sites were grafted with rhPDGF-BB + β-TCP.

CTG results: tooth #21. Figure 7 illustrates the surgical timeline of this study. Two months after creation of the recession defect (Figs. 7A and 7B), osseous and gingival margin reference notches were placed at the buccal osseous crest and precorrected gingival margin and the area grafted with a CTG from the palate. Then a buccal flap was coronally advanced superior to the CTG; the outcome at 9 months is seen in Figure 7C. A conservative en bloc resection was

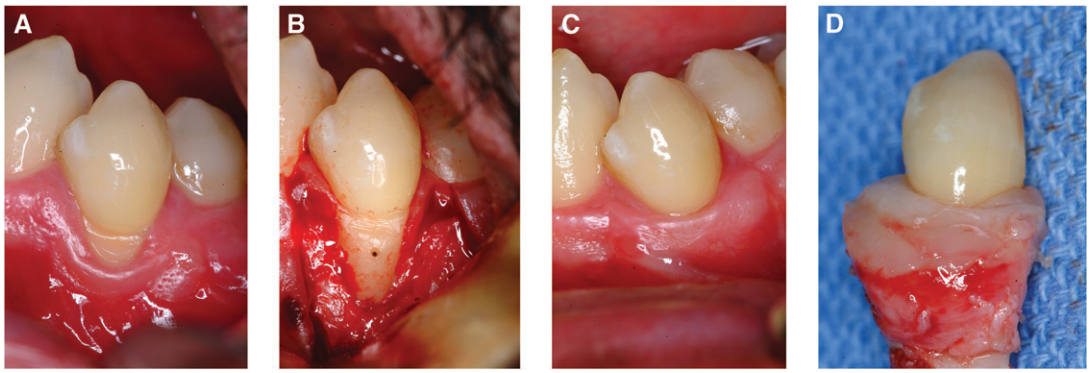

Figure 7.
A) *Clinical photograph of gingival recession defect 2 months after its surgical creation. Osseous and gingival reference notches were placed at the time of surgical correction of the recession defect.* ***B)*** *In each case, the osseous crest was placed ~3 mm apical to the precorrected gingival margin.* ***C)*** *100% root coverage is maintained 9 months after correction of the recession defect with the CTG.* ***D)*** *Conservative en bloc resection of tooth #21 along with adjacent hard and soft tissues.*

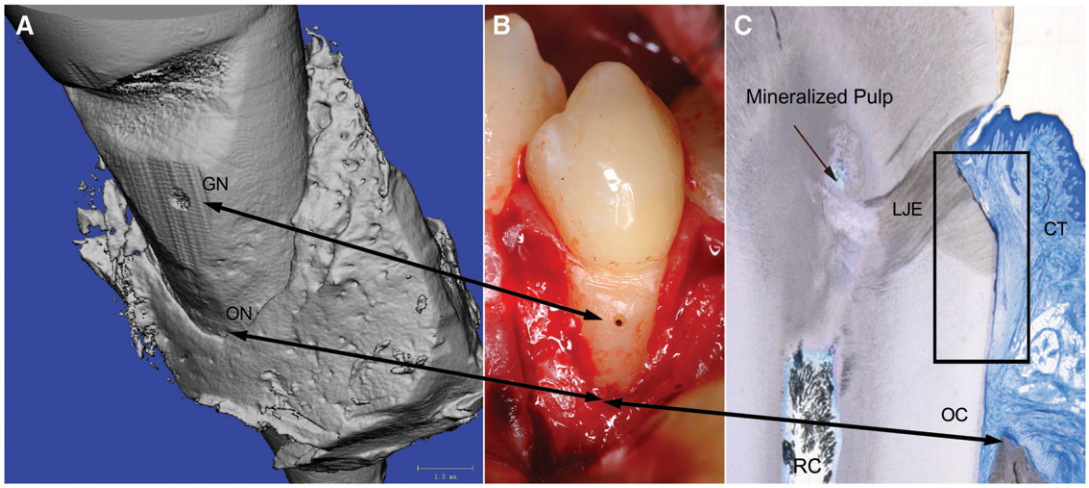

Figure 8.
A) *A micro-CT image of the en bloc resection obtained 9 months after CTG correction of the recession defect. The bottom arrow represents the osseous reference notch (ON), and the top arrow denotes the gingival margin reference notch (GN). No evidence of bone regeneration is evident 9 months after connective tissue grafting.* ***B)*** *Osseous and gingival margin reference notches were placed at the time of surgical correction of the recession defect.* ***C)*** *Histologic ground section confirms no evidence of periodontal regeneration 9 months after connective tissue grafting. Box: An LJE is seen extending just coronal to the original osseous crest along with abundant non-inserting connective tissue (CT) fibers. OC = osseous crest; RC = root canal. (Toluidine blue/Azur II; original magnification: ×12).*

performed at 9 months (Fig. 7D) for microscopic ground section and micro-CT examination.

Nine months after CTG, no evidence of periodontal regeneration was evident. Figure 8A is a micro-CT image of the en bloc resection obtained at 9 months following CTG correction of the recession defect. The bottom arrow points to the osseous reference notch, and the top arrow denotes the gingival margin reference notch. Neither notch is obscured with overlying regenerated bone. As noted in Figures 8A and 8B, no change in the level of the osseous crest occurred during the 9-month period after CTG grafting. Ground-section histologic examination at 9 months (Fig. 8C) confirmed the micro-CT findings. An LJE extends apically, ending just superior to the original osseous crest. Abundant connective tissue without evidence of bone or cementum regeneration is seen coronal to the osseous crest. A bucco-lingual micro-CT view emphasizes the complete lack of bone regeneration seen in the en bloc resected specimen 9 months after CTG + CAF treatment (Fig. 9A). This lack of bone regeneration was also demonstrated in an additional bucco-lingual micro-CT image through the central portion of the tooth; no bone regeneration is seen coronal to the osseous notch (Fig. 9B).

rhPDGF-BB + β-TCP graft results: tooth #12. As in the above CTG specimen, Figure 10 represents the identical surgical timeline, except that this site was

grafted with rhPDGF-BB + β-TCP + a collagen wound-healing dressing with an overlying CAF. As in the former grafted site, the osseous crest was placed ~3 mm apical to the precorrected gingival margin.

Nine months after grafting with rhPDGF-BB + β-TCP, micro-CT revealed substantial bone regeneration beginning at the osseous notch and extending coronally toward the gingival reference notch (Fig. 11). The regenerated tissue appears as dense cortical bone analogous to the lamina dura observed in dental periapical radiographs.

Further identification of the osseous and gingival reference notches, critical to determining the presence and extent of periodontal regeneration, is seen in additional sagittal micro-CT imagery (Fig. 12). Bone regeneration coronal to the osseous notch is readily appreciated in this micro-CT image.

Ground mineralized histologic sections, used in this study for increased clarity over demineralized serial sections, confirmed and amplified the micro-CT results, demonstrating robust coronal bone and cementum regeneration with a uniformly dimensioned PDL space 9 months after grafting with rhPDGF-BB + β-TCP (Fig. 13). The osseous reference notch is well defined in the sagittally positioned ground section (Fig. 13B). Note the partial occlusion of the osseous notch by newly regenerated cementum. Residual β-TCP particles seen in both ground sections appear to be inhibiting the more robust bone regeneration seen in areas where the ceramic has already resorbed.

To better demonstrate and define the absolute position of each reference notch, multiple horizontal-CT sections were taken, with 18 μm between cuts, beginning at the most apical extent of the imaged en bloc resection. Micro-CT cuts 339 and 527 correspond to the locations of the osseous and gingival reference notches, respectively, representing an inter-notch distance of 188 micro-CT horizontal sections or ~3.4 mm (Figs. 12, 14, and 15).

Under higher magnification of the ground section in Figure 13A, significant coronal bone

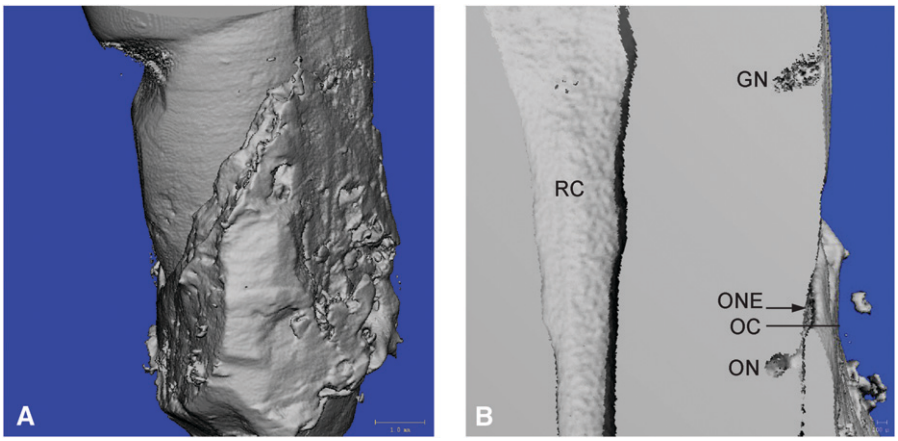

Figure 9.
A) *Bucco-lingual micro-CT image reveals no evidence of bone regeneration coronal to the osseous reference notch 9 months after correction of the recession defect with a CTG and CAF.* ***B)*** *Bucco-lingual micro-CT image through the central portion of tooth #21 demonstrates no bone regeneration coronal to the osseous notch (ON) at the tooth surface. A small amount of preexisting interproximal bone is seen above the osseous notch entry point (ONE) secondary to slight rotation of the image. OC = osseous crest; RC = root canal; GN = gingival notch.*

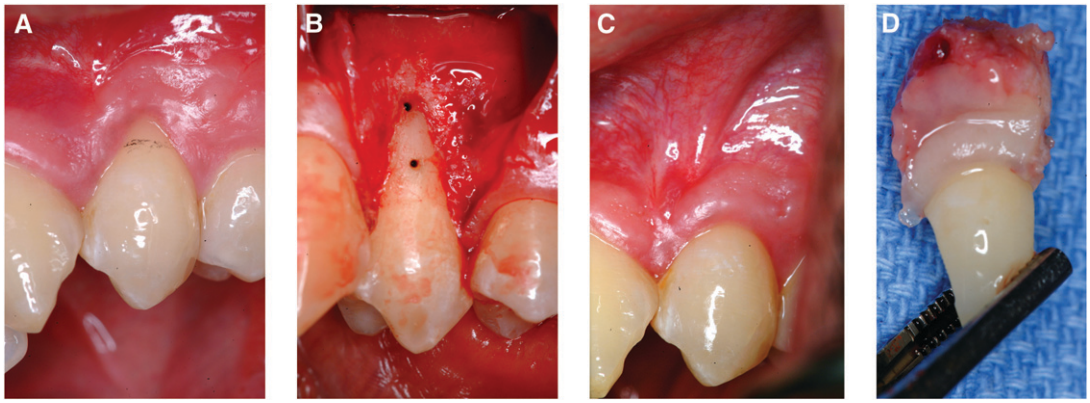

Figure 10.
A) *Clinical photograph of gingival recession defect 2 months after its surgical creation.* ***B)*** *Osseous and gingival reference notches placed at the time of surgical correction of the recession defect. In each case the osseous crest was placed ~3 mm apical to the precorrected gingival margin.* ***C)*** *One hundred percent root coverage is maintained 9 months after correction of the recession defect with rhPDGF-BB + β-TCP.* ***D)*** *Conservative en bloc resection of tooth #12 along with adjacent hard and soft tissues.*

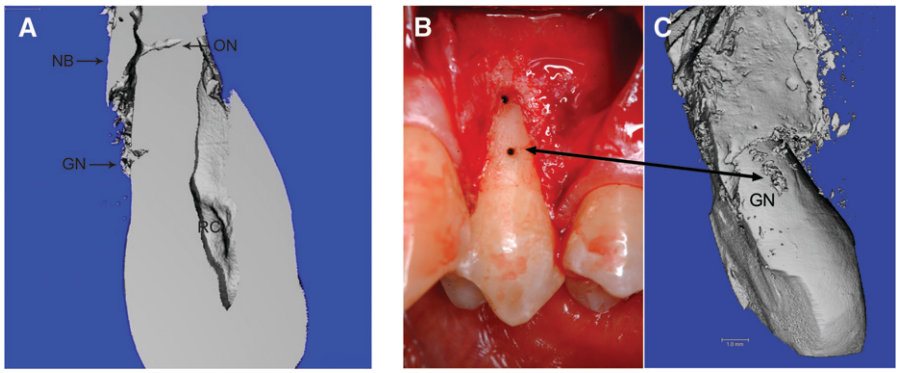

Figure 11.
*Nine months after grafting with rhPDGF-BB + β-TCP, sagittal **(A)** and coronal **(C)** micro-CT reveal coronal bone regeneration superior to the osseous notch **(B)**. The regenerated tissue appears as dense cortical bone analogous to the lamina dura observed in dental periapical radiographs. ON = osseous notch; NB = new bone; GN = gingival notch; RC = root canal.*

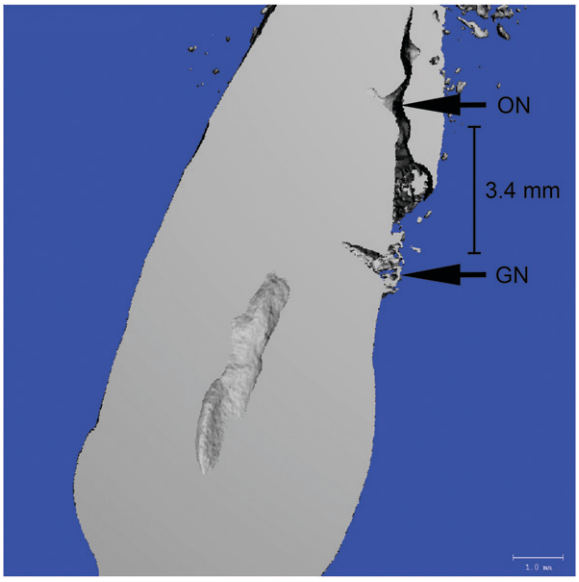

Figure 12.
Osseous and gingival reference notches are clearly seen in this sagittal micro-CT image as well as bone regeneration coronal to the osseous notch. The distance between notches is ~3.4 mm. ON = osseous notch; GN = gingival notch.

regeneration is seen (Fig. 16). In addition, regeneration of cellular cementum as well as a uniform PDL space between the newly formed bone and the adjacent tooth surface are seen.

At still higher magnification, all three tissues of the normal periodontium, i.e., cementum, PDL, and supporting alveolar bone, are seen in greater detail (Figs. 17 and 18). Osteocytes and cementocytes are clearly seen in newly formed bone and cementum, respectively. It is significant that connective tissue fibers of the newly regenerated PDL insert directly into newly formed cementum and bone. Polarized light imagery confirms the ground section finding that connective tissue fibers insert directly into newly regenerated adjacent tissue (Fig. 19).

DISCUSSION

To examine therapeutic effectiveness when addressing gingival recession defects, researchers and clinicians must determine whether any given therapy successfully addresses the major components of these complex lesions. The following are common to all recession defects: loss of the protective functional morphology of the mucogingival complex, an esthetic imbalance among marginal tissues and the adjacent tooth root and crown, and anatomic loss of portions of the attachment apparatus, i.e., cementum, PDL, and supporting alveolar bone.[1] Comprehensive treatment successfully addresses each of these components. The current study, divided into two sections, examined and compared the safety and efficacy of the CTG to a novel tissue-engineered approach on each of the major components of recession defects.

Multiple studies,[2-5] including two systematic reviews of randomized, controlled clinical trials, verified the long-term efficacy of the CTG with a CAF in maintaining root coverage. However, unlike past studies, the current trial compared the CTG to a therapeutic approach using rhPDGF-BB + β-TCP + a collagen wound-healing dressing, each covered with a CAF. In both therapies, clinically significant improvements from baseline were observed at the 24-week postoperative visit in recession depth reduction; CAL gain; PDRs at buccal, mesial, or distal surfaces; increases in the height of keratinized tissue; root coverage; and recession width reduction. There were no statistically significant differences observed between the two treatments for all efficacy endpoints measured, except for the reductions in recession depth, recession width, and root coverage, each favoring the subepithelial CTG treatment; PDR at the mid-buccal surface statistically favored the rhPDGF-BB + β-TCP treatment. No statistically significant differences were seen between the two treatments in esthetically related endpoints, i.e., color/texture of the tissues, as determined by the esthetic satisfaction questionnaire. In terms of overall satisfaction, no differences were seen between the two treatment modalities. At the conclusion of the study, a number of patients required additional surgery to correct recession defects adjacent to other teeth. Each patient requiring additional surgery was given a choice between CTG or

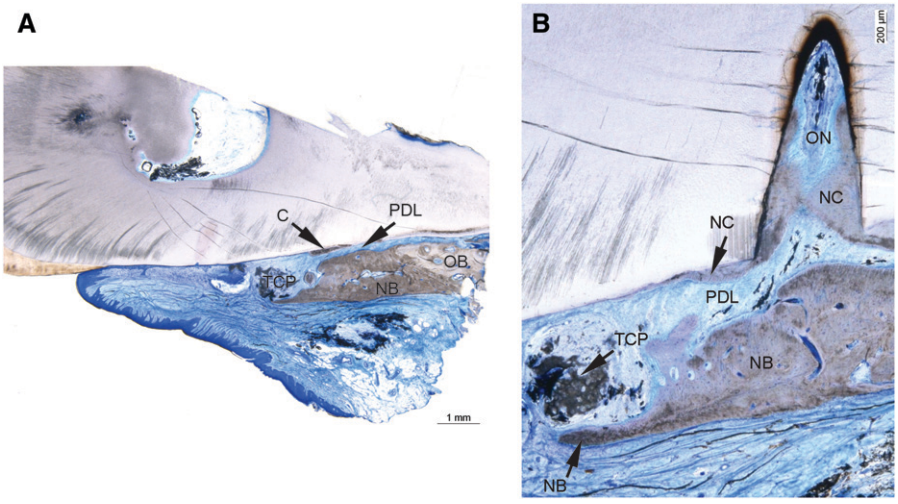

Figure 13.
A) *At low power, a ground section demonstrates robust coronal bone regeneration 9 months after grafting with rhPDGF-BB + β-TCP. Note the uniform PDL space between newly formed bone (NB) and the adjacent tooth surface as well as the formation of new cementum (C). Coronally, residual TCP particles appear to inhibit the amount of bone regeneration seen elsewhere in the specimen. OB = old bone.* ***B)*** *Newly formed cementum (NC) is seen occluding the orifice of the osseous notch (ON) in this higher power sagittal ground section. New bone (NB) and cementum have formed coronal to the osseous notch. (Toluidine blue/Azur II; original magnification: A, ×7; B, ×20.)*

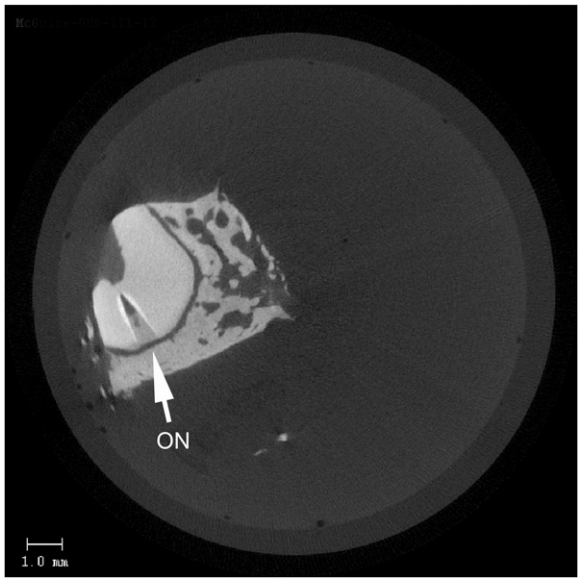

Figure 14.
Horizontal micro-CT image at level 339 clearly demonstrating the location of the osseous reference notch (ON; arrow).

rhPDGF-BB–mediated treatment; all chose the rhPDGF-BB + β-TCP treatment. Two main reasons were given: the subjective esthetic results were equivalent, and the treatment with the growth factor did not require an additional harvesting procedure.

Although the RCT portion of this study examined efficacy parameters related to restoring the protective functional morphology of the mucogingival complex as well as the esthetic balance between marginal tissues and the adjacent tooth root and crown, a histologic examination was required to assess the ability of each therapeutic modality to effectively regenerate missing portions of the attachment apparatus. To compare the periodontal regenerative potential of each therapy, recession defects with well-defined anatomic characteristics were surgically created buccal to six teeth planned for extraction secondary to orthodontic treatment.

Unlike traditional GTR procedures, barrier membranes were not used in this study. Accepted recession-related treatment with CTGs does not call for barrier membranes; therefore, none were used. In the test group, in lieu of a barrier membrane, a porous collagen wound-healing dressing[#] saturated with rhPDGF-BB was placed over the β-TCP + rhPDGF-BB grafts. The collagen dressing helped to prevent β-TCP particle migration and provided an additional source for the release of rhPDGF-BB into the surrounding environment. Unlike traditional GTR procedures, which exclude unwanted cell migration into an underlying grafted site, growth factor–mediated treatment chemotactically seeks out mesenchymal stem cells, preosteoblasts, and fully committed osteoblasts that are found in large numbers within the overlying periosteum.[21-23] Unlike barrier membranes, which would prevent the active migration of these latter cells into the grafted site from the overlying periosteum, a porous wound-healing dressing likely permits such entrance into the grafted area through directed growth factor–mediated cell migration.

Nine months after surgical correction of the defects with a CTG or rhPDGF-BB + β-TCP, conservative en bloc resections were performed, and each specimen was studied histologically and through micro-CT examination. Clear and significant differences were seen in each treatment's ability to regenerate new cementum with inserting connective tissue fibers and supporting alveolar bone.

Two of six sites were treated with CTGs, and neither exhibited signs of periodontal regeneration. In neither site was there any evidence of cementum, PDL, or

CollaTape, Integra LifeSciences.

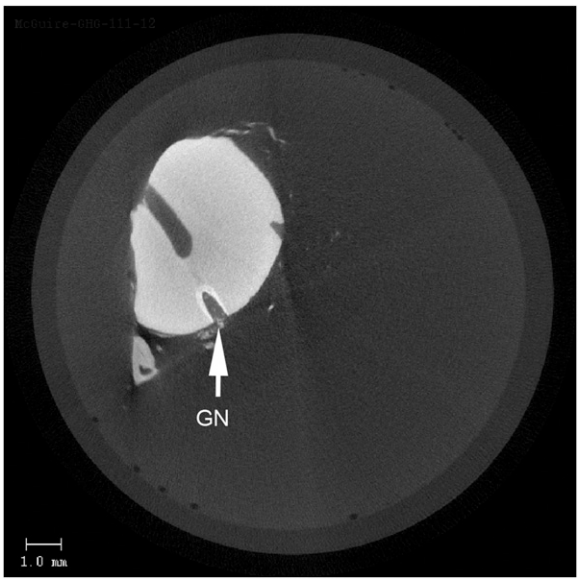

Figure 15.
Identification of the gingival reference notch (GN; arrow) at horizontal micro-CT image level 527 represents a distance ~3.4 mm from the osseous notch.

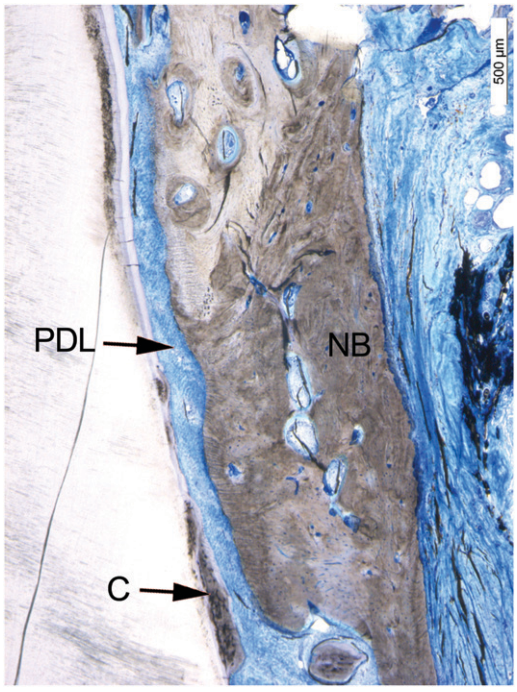

Figure 16.
Higher magnification reveals significant new bone (NB) formation following grafting with rhPDGF-BB + β-TCP. Also note the well-developed PDL space and the regenerated area of newly formed cementum (C). (Toluidine blue/Azur II; original magnification: ×18.)

supporting bone regeneration. In both instances, an LJE extended just coronal to the original osseous crest. In addition, although an abundant amount of connective tissue formed, collagen fibers generally ran parallel

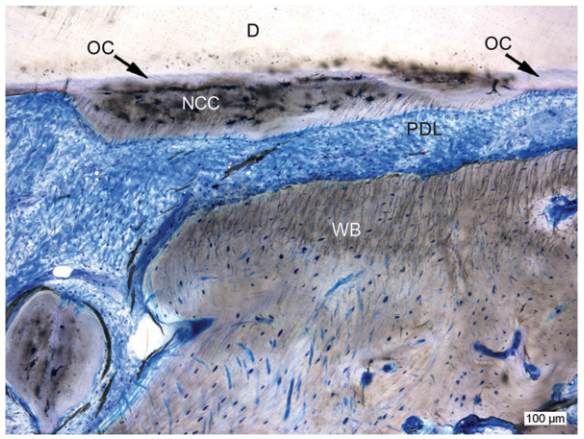

Figure 17.
High-power ground section view of tooth #12 reveals regeneration of all tissues of the missing periodontium. New cementum is highly cellular. Cementocytes and osteocytes are seen in regenerated cementum and bone, respectively. OC = old cementum; D = dentin; NCC = new cellular cementum; WB = woven bone. (Toluidine blue/Azur II; original magnification: ×45.)

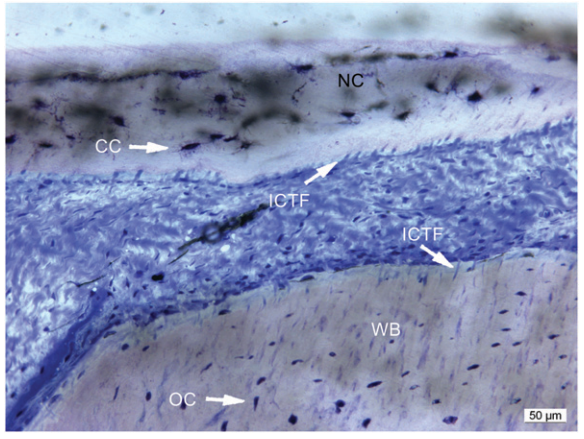

Figure 18.
At higher magnification, cementum, PDL, and alveolar bone are seen in greater detail. Osteocytes (OC) and cementocytes (CC) are clearly seen in newly formed bone and cementum, respectively. Connective tissue fibers of the newly regenerated PDL are seen inserting directly into newly formed cementum and bone. NC = new cementum; ICTF = inserting connective tissue fibers; WB = woven bone. (Toluidine blue/Azur II; original magnification: ×75.)

to the adjacent root surfaces, with no evidence of inserting Sharpey's fibers.

In contrast, the representative site, as well as the other three sites grafted with 0.3 mg/ml rhPDGF-BB + β-TCP, exhibited definitive signs of periodontal regeneration, i.e., formation of new cementum, PDL, and supporting bone. In the present example, multiple histologic ground sections demonstrated significant new bone growth coronal to the osseous reference notch. Robust regeneration of cellular cementum occurred

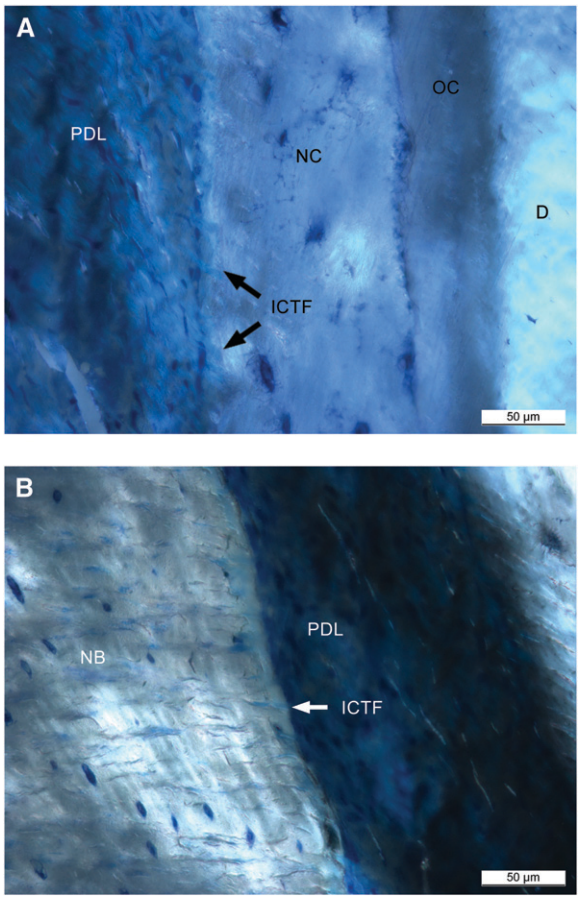

Figure 19.
*Polarized light confirms that connective tissue fibers insert directly into newly regenerated cementum **(A)** and bone **(B)**. ICTF = inserting connective tissue fibers; NC = new cementum; OC = old cementum; D = dentin; NB = new bone. (Toluidine blue/Azur II; original magnification: ×90.)*

coronal to the osseous notch in this and the other three sites grafted with rhPDGF-BB + β-TCP. High-power magnification under polarized and non-polarized light demonstrated PDL collagen fibers inserting directly into newly regenerated cementum and bone.

In addition to light microscopy, micro-CT was used to examine and infer the type of attachment obtained following CTG or growth factor–mediated treatment of these surgically created recession defects. Recently, Nevins et al.[24] used micro-CT technology to evaluate periodontal regeneration in the treatment of intrabony defects. Using micro-CT, the investigators were able to evaluate a number of parameters, including the two- and 3D anatomy of preexisting and newly regenerated bone, the thickness and 3D shape of the PDL space, and the suggestion of regeneration as defined by the micro-CT findings of adjacent new bone connected by a PDL space in relation to a reference root notch. Likewise in this study, micro-CT evaluation confirmed information gained from histologic ground sections and yielded unambiguous information confirming the presence or absence of bone regeneration coronal to the osseous reference notch. Based on micro-CT findings alone, grafting with rhPDGF-BB + β-TCP led to significant coronal bone regeneration, whereas grafting with CTGs led to none. In addition, micro-CT evaluation suggested that at 9 months postgrafting, the growth factor–mediated bone appeared as dense cortical bone, similar to that seen as the lamina dura in periapical dental radiographs. Finally, residual particles of β-TCP inferior to the newly regenerated bone appeared to diminish the robust bone formation seen in areas where the ceramic had already resorbed (Fig. 13). According to Ridgway et al.,[17] residual particles of β-TCP may delay PDGF-mediated bone formation.

CONCLUSIONS

The CTG and growth factor–mediated treatment modalities led to clinically effective outcomes for the correction of gingival recession defects. Although changes in recession depth, width, and percentage of root coverage statistically favored the CTG at 6 months, the subjects' esthetic satisfaction was the same for both treatments. The tissue-engineered approach had the added advantage of requiring no harvesting procedure. Histologically, none of the CTG-treated sites yielded evidence of periodontal regeneration, whereas the combination of rhPDGF-BB + β-TCP + wound-healing dressing consistently led to the formation of new cementum with inserting connective tissue fibers and supporting alveolar bone in all four treated defects. To our knowledge, this is the first published study to document human histologic evidence of regeneration in the treatment of recession defects using recombinant growth factor technology.

In this study, surgically created recession defects were used to examine histologic and micro-CT results. Exposed root surfaces were allowed time to become contaminated to simulate naturally occurring gingival recession defects. Although difficult because of the need to obtain en bloc resections, additional histologic studies using long-standing recession defects would add to the body of knowledge and further define the regenerative potential of growth factor–mediated treatment. Finally, additional studies examining carriers for rhPDGF-BB, other than β-TCP, would provide added insight into the potential effectiveness of tissue-engineered solutions for the treatment of gingival recession defects.

ACKNOWLEDGMENTS

The authors acknowledge the following individuals for their help in this project: Carol Waring, registered dental hygienist (RDH) and study coordinator, Rebecca Garcia, RDH and director of clinical research, Cindy Wainscott, certified dental assistant and office

manager, and Dr. Jacquelyn Campbell, editor, Perio Health Professionals, Houston, Texas, for assistance with conducting the study and developing the manuscript; Jennifer Gramazio, marketing assistant, Osteohealth, Shirley, New York, for her assistance with the images; Dr. Myron Nevins, private practice in periodontics, Swampscott, Massachusetts, for his assistance with the histology; Dr. Marc Nevins, private practice in periodontics, Boston, Massachusetts, for his guidance with the block sections; Mr. Leigh Ren, statistician, Target Health, New York, New York, for assistance with the statistical analysis; Dr. Palle Holmstrup, Department of Periodontology, Royal Dental College, Copenhagen, Denmark, for his histologic review; and special thanks to Dr. Stuart Kay, science writer and consultant, Huntington, New York, for his help with the organization and production of the manuscript. This study was supported by a grant from Osteohealth, a division of Luitpold Pharmaceuticals, Shirley, New York. The rhPDGF-BB and β-TCP used in this study are marketed and sold by Osteohealth. Drs. McGuire and Scheyer have received lecture fees from Osteohealth. Dr. Schupbach reports no financial relationships related to any products or commercial firms involved in this study.

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Submitted October 2, 2008; accepted for publication December 5, 2008.