# Biologics-based Regenerative Technologies for Periodontal Soft Tissue Engineering

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**Running Title:** Biologics-based technologies for root coverage

**One Sentence Summary:** This review highlights the current evidence on the use of biologics-based technologies for periodontal soft tissue wound repair

**Abstract**

This manuscript provides a state-of-the-art review on the efficacy of biologics in root coverage procedures, including enamel matrix derivative, platelet-derived growth factor, platelet concentrates, and fibroblast-growth factor-2. The mechanism of action and the rationale for using biologics in periodontal plastic surgery, as well as their anticipated benefits when compared to conventional approaches are discussed. Although the clinical significance is still under investigation, preclinical data and histological evidence demonstrate that biologic-based techniques are able to promote periodontal regeneration coupled with the provision of tooth root coverage.

***Key words:*** gingival recession, growth factors, periodontal, regeneration, soft tissue grafting, tissue engineering

**Biologics-based Regenerative Technologies**

Gingival recession is defined as the apical migration of the gingival margin from the cemento-enamel junction with the concomitant exposure of the root surface in the oral cavity [1]. This condition also involves the resorption of the facial alveolar bone and associated structures. Therefore, it's not surprising that guided tissue regeneration (GTR) was one of the first approaches that was proposed for the treatment of gingival recessions [2-4]. However, the use of GTR for root coverage purposes has had several limitations, including limited predictability in cases of shallow recession defects or cases of thin gingival thickness [5]. Thus, several authors have explored the use of biologic agents or growth factors, which are group of proteins capable to induce gene or cell activations for cell recruitment, matrix biosynthesis and cellular differentiation, in the attempt to regenerate the lost periodontium [50, 6-10]. This review aims to present the mechanism of action, the rational and outcomes of biologics-based regenerative technologies for the promotion of tooth root coverage.

**Enamel matrix derivative (EMD)**

Enamel matrix proteins are deposited on the developing tooth roots prior to the formation of the cementum [11, 12]. It has been shown that EMD obtained from porcine fetal tooth EMD** biomimetically stimulates cementogenesis by enhancing proliferation and migration of periodontal ligament cells (PDL) and osteoblasts, mimicking the natural process of tooth development [13-15]. While EMD has been initially proposed for periodontal regeneration, these proteins have also been investigated for root coverage procedures (Figure 1) and soft tissue healing [13] given its properties of enhancing vasculogenesis and local growth factor expression [13, 16, 17]. However, whether or not EMD adds clinical benefits in the treatment of gingival recessions (GRs) remains unclear. Modica and coworkers demonstrated a greater mean root coverage and clinical attachment level (CAL) gain when EMD was used in combination with coronally advanced flap (CAF) when compared to CAF

alone [18]. A multi-center study showed that the outcomes of root coverage procedure by using CAF + CTG can be further enhanced by the addition of EMD [6]. However, other studies did not demonstrate a significant enhancement when using EMD in terms of greater root coverage results [7, 19-21], leading Del Pizzo et al. to conclude that the addition of EMD to CAF is not justified for clinical benefits of root coverage, but as an attempt to obtain periodontal regeneration [7]. Indeed, an *in-vivo* histological study by McGuire & Cochran showed a site that received CAF + CTG healed with long-junctional epithelium and connective tissue attachment at the root surface, while new cementum, interspersed connective tissue (interpreted to be organizing periodontal ligament, PDL), and islands of condensing bone found at a fixed distance from the root surface was observed in the histological section of CAF + EMD [8]. The property of EMD to induce periodontal regeneration in GRs was later corroborated in a prospective case-control study involving a histological and micro-CT analysis by McGuire and co-workers [22] (Figure 2). A recent randomized clinical trial evaluating the efficacy of several root coverage procedures (CAF alone, CAF + EMD, CAF + collagen matrix and CAF + collagen matrix + EMD) showed that CAF + collagen matrix + EMD was related to the greater percentage of complete root coverage [23]. Similarly, other studies have suggested that combining allografts to EMD may enhance the clinical outcomes [24-26], probably due to the scaffold role of the graft material that contributes to the stability of the wound and maintains the space necessary for periodontal regeneration. In addition, the potential role of EMD in improving the esthetic results and patient satisfaction has been reported [27, 28]. Lastly, it should be mention that the addition of biologics has been found to be beneficial in smokers [24, 26].

**Platelet-derived growth factor-BB (PDGF)**

Platelet-derived growth factor-BB (PDGF) is one of most investigated growth factors in periodontal tissue engineering. Since its early introduction in the late 1980s [29], several animal and clinical studies have confirmed its role in promoting bone, cementum and PDL regeneration [29-32]. Regarding its

mechanism of action, it has been shown that PDL and alveolar bone cells express multiple receptors α, β, χ, δ for PDGF, which enhances proliferation and chemotaxis of these cells [30, 33-35]. In particular, Boyan et al. investigated the effect of the various isoforms of recombinant human PDGF (AB, AA and BB) on the mitogenic and chemotactic responses of PDL cells, showing that PDGF-BB was the most potent one [36]. McGuire & Scheyer were the first to investigate the use of rhPDGF-BB for the treatment of GRs in a case series involving the use of connective tissue graft (CTG) as controls[9]. After flap elevation, rhPDGF-BB solution was applied to the exposed root surfaces and to the coronal PDL fibers, and then a small amount of β-tricalcium phosphate (β-TCP) saturated with the rhPDGF-BB solution was placed over the root. A collagen matrix was saturated with the rhPDGF-BB solution and adapted over the graft before the suturing of the flap. The rationale of using β-TCP as a carrier of the rhPDGF-BB†† is that β-TCP particles have a scaffolding role preventing the soft tissue flap collapse against the root surface and providing a matrix for the new bone formation, facilitating also the stabilization of the blood clot. The treatment was found to be effective in terms of root coverage and esthetic outcomes, with no patients reporting adverse events. These findings led the authors to conclude that this case series provided proof-of-principle for the treatment of GRs with rhPDGF-BB plus β-TCP and a collagen matrix without the need for autogenous CTG harvested from the palate [9].

Based on these promising results, the same group designed a split-mouth randomized clinical trial comparing coronally advanced flap + rhPDGF-BB + β-TCP + bioabsorbable collagen wound-healing dressing (test group) to CAF + CTG [10]. Although the controls showed superior recession depth reduction after 9 months, the growth factor-treated sites displayed greater PD reduction at the study completion. Similar KTW gain, esthetic results and patient satisfaction was observed for both groups. In addition, the study included a histologic and micro-CT analysis of 6 treated teeth requiring extraction for orthodontic therapy. After 9 months, while CAF + CTG group showed healing with long-junctional epithelium and connective tissue fibers running parallel to the root surface, the growth factor-treated sites showed evidence of periodontal regeneration. Regenerated bone was

visualized coronal to the notch by micro-CT evaluation, while the histological analysis showed osteocytes and cementocytes entombed in newly formed bone and cementum. The newly regenerated PDL exhibited Sharpey fibers obliquely inserting into the newly formed cementum and bone. The study demonstrated that a correction of the GRs together with regeneration of the periodontium can be obtained using a PDGF-mediated approach [10] (Figure 3).

Subsequent studies using rhPDGF-BB, combined to β-TCP and collagen matrix [37] were compared to ADM [38] or to CTG [39] for root coverage applications. Deshpande and coworkers obtained a mean root coverage of 91.3% and 87.7% using CTG and rhPDGF-BB + β-TCP + collagen membrane, respectively, while CAF alone achieved 68.6% of mean root coverage at 6 months [40].

Although the use of rhPDGF-BB may achieve comparable outcomes with autologous CTG in the treatment of GRs, there is no evidence that a regenerated recession with new bone, cementum and PDL has a greater long-term stability than a recession treated with flap alone or CTG, which exhibit healing by repair. In a 5-year follow-up study, McGuire et al. showed good stability for 5 years as compared to the initial 6-month time point in terms of recession depth changes between CTG and rhPDGF-BB groups. However, CTG showed superior results in recession reduction, percentage of sites with complete root coverage and KTW gain [41].

**Platelet concentrates**

A patient's own blood has been centrifugated in multiple fields of medicine to concentrate platelets in an attempt to increase the density of growth factors and potential enhance wound healing for soft and hard tissues.[50] Platelet concentrates (PCs) have been considered as wound healing promoters for infrabony defects and sinus floor elevation [42, 43] as well as uses as a scaffold matrix in root coverage procedures [44, 45]. Platelet-rich plasma (PRP) and plasma rich in growth factor (PRGF) are considered the first generation of PCs [46]. Their impact on root coverage outcomes has been shown

to be minimal and in general, non-significant[47-49]. The biologic limitation of autogenous platelet concentrates is that the GF composition is orders of magnitude lower than can be achieved with recombinant GFs such as PDGFs and FGFs. As such, it remains questionable that biologically relevant concentrations to promote significant regeneration can be achieved with these procedures [50].

The second generation of PCs involves the centrifugation of the blood without the addition of anticoagulants [46]. Based on the processing speed and duration, Platelet-rich fibrin (PRF) is classified in A-PRF, L-PRF and titanium prepared PRF (T-PRF). Among its advantages, the released of growth factors, including PDGF, vascular endothelial growth factors (VEGF), TGF β-1 and Insulin-link growth factors (IGF-1), has been described [51, 52]. In root coverage procedures, PRF has been investigated alone [45], in combination with tissue grafts [53] or as wound healing enhancer in the palatal donor site after harvesting procedures [54, 55]. Nevertheless, it seems that the addition of PRF did not further enhance the outcomes of the root coverage procedure [53] and that autologous connective tissue graft remains the technique of choice [56, 57]. A possible explanation is that gingival recessions benefit from the thickening of the soft tissue margin and PRF membranes do not act as scaffolds promoting the migration of cells from adjacent tissues. In addition, early studies demonstrated that higher concentration of factors typically expressed by platelets, such as IL-1β and TGF-β, resulted in an inhibitory interaction on PDGF-AA by decreasing the number of PDGF-α receptors and available binding sites, which may also explain the limited success of platelet concentrates for root coverage [58, 59]. In summary, the use of platelet concentrates containing insignificant enrichment of GF levels have failed to demonstrate significant enhancements in wound repair and root coverage. As such, the clinical usage of these procedures remains is not recommended based on current evidence.

**Fibroblast-growth factor-2**

Fibroblast growth factor-2 (FGF-2) is a heparin-biding cytokine which is able to enhance the angiogenic and osteogenic activity of multiple cellular populations [60-62]. In addition, FGF-2 can stimulate the proliferation and migration of mesenchymal cells within the PDL [62]. Given these strong wound healing characteristics, FGF-2 has been thoroughly explored in periodontal and bone regeneration, alone or in combination with scaffolding matrices [61, 63-65]. Ishii and coworkers were the first to evaluate the effect of FGF-2 on root coverage outcomes in vivo [66]. In a split-mouth randomized design, recession defects were created bilaterally and assigned to the treatment FGF-2 alone or FGF-2 combined with a carrier matrix (β-TCP). Microscopic and histometric analysis showed that both approaches were effective to inhibit epithelial down-growth while achieving some periodontal regeneration. In particular, FGF-2 + β-TCP exhibited a greater amount of new bone and cementum formation than FGF-2 alone, suggesting that the GF benefits in combination with a scaffold that allows cellular and vascular invasion, migration and growth [66]. When GR occurs, the facial alveolar bone is resorbed resulting in an unfavorable defect architecture for regeneration [61, 66, 67]. Therefore, it can be speculated that a scaffold provides better stability to the blood clot and GF. Consistent with these findings, two recent preclinical studies showed superior outcomes when FGF-2 was incorporated into the scaffolds compared to FGF-2 alone [61, 68]. Enhanced new cementum and bone was observed in the site treated with FGF-2 and a biodegradable sponge [61], while another group found that a collagen matrix scaffold was able to enhance the amount of root coverage of FGF-2 at 4 and 16 weeks [68]. These preclinical studies showed that FGF-2, especially when incorporated into a scaffold enhances periodontal regeneration and root coverage. Nevertheless, human clinical trials are necessary to validate these preclinical findings.

At this moment, biologic-based technologies have not yet been applied to implant dehiscence defect soft tissue coverage.

***Concluding remarks***

There is evidence supporting the use of EMD or PDGF for root coverage when the aim is to treat gingival recessions while correspondingly promoting periodontal regeneration. Platelet concentrates have demonstrated equivocal results and their clinical usage remains to be further determined. Preclinical data using FGF-2 are encouraging and human studies are required to determine the potential for clinical use. Future development of the use of biologics for soft tissue regeneration may involve coupling growth factors with next-generation bioresorbable scaffolds to augment both alveolar bone and soft tissue.

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## Footnotes

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**References**


1. Cortellini P, Bissada NF. Mucogingival conditions in the natural dentition: Narrative review, case definitions, and diagnostic considerations. *J Clin Periodontol* 2018;45 Suppl 20:S190-S198.

2. Pini Prato G, Tinti C, Vincenzi G, Magnani C, Cortellini P, Clauser C. Guided tissue regeneration versus mucogingival surgery in the treatment of human buccal gingival recession. *J Periodontol* 1992;63:919-928.

3. Tinti C, Vincenzi G, Cortellini P, Pini Prato G, Clauser C. Guided tissue regeneration in the treatment of human facial recession. A 12-case report. *J Periodontol* 1992;63:554-560.

4. Kimble KM, Eber RM, Soehren S, Shyr Y, Wang HL. Treatment of gingival recession using a collagen membrane with or without the use of demineralized freeze-dried bone allograft for space maintenance. *J Periodontol* 2004;75:210-220.

5. Wang HL, Modarressi M, Fu JH. Utilizing collagen membranes for guided tissue regeneration-based root coverage. *Periodontol 2000* 2012;59:140-157.

6. Rasperini G, Roccuzzo M, Francetti L, Acunzo R, Consonni D, Silvestri M. Subepithelial connective tissue graft for treatment of gingival recessions with and without enamel matrix derivative: a multicenter, randomized controlled clinical trial. *Int J Periodontics Restorative Dent* 2011;31:133-139.

7. Del Pizzo M, Zucchelli G, Modica F, Villa R, Debernardi C. Coronally advanced flap with or without enamel matrix derivative for root coverage: a 2-year study. *J Clin Periodontol* 2005;32:1181-1187.

8. McGuire MK, Cochran DL. Evaluation of human recession defects treated with coronally advanced flaps and either enamel matrix derivative or connective tissue. Part 2: Histological evaluation. *J Periodontol* 2003;74:1126-1135.

9. McGuire MK, Scheyer ET. Comparison of recombinant human platelet-derived growth factor-BB plus beta tricalcium phosphate and a collagen membrane to subepithelial connective tissue grafting for the treatment of recession defects: a case series. *Int J Periodontics Restorative Dent* 2006;26:127-133.

10. McGuire MK, Scheyer ET, Schupbach P. Growth factor-mediated treatment of recession defects: a randomized controlled trial and histologic and microcomputed tomography examination. *J Periodontol* 2009;80:550-564.

11. Lindskog S, Hammarstrom L. Formation of intermediate cementum. III: 3H-tryptophan and 3H-proline uptake into the epithelial root sheath of Hertwig in vitro. *J Craniofac Genet Dev Biol* 1982;2:171-177.

12. Slavkin HC, Bessem C, Fincham AG, et al. Human and mouse cementum proteins immunologically related to enamel proteins. *Biochim Biophys Acta* 1989;991:12-18.

13. Miron RJ, Sculean A, Cochran DL, et al. Twenty years of enamel matrix derivative: the past, the present and the future. *J Clin Periodontol* 2016;43:668-683.

14. Hoang AM, Oates TW, Cochran DL. In vitro wound healing responses to enamel matrix derivative. *J Periodontol* 2000;71:1270-1277.

15. Yoneda S, Itoh D, Kuroda S, et al. The effects of enamel matrix derivative (EMD) on osteoblastic cells in culture and bone regeneration in a rat skull defect. *J Periodontal Res* 2003;38:333-342.

16. Tonetti MS, Fourmousis I, Suvan J, et al. Healing, post-operative morbidity and patient perception of outcomes following regenerative therapy of deep intrabony defects. *J Clin Periodontol* 2004;31:1092-1098.

17. Maymon-Gil T, Weinberg E, Nemcovsky C, Weinreb M. Enamel Matrix Derivative Promotes Healing of a Surgical Wound in the Rat Oral Mucosa. *J Periodontol* 2016;87:601-609.

18. Modica F, Del Pizzo M, Roccuzzo M, Romagnoli R. Coronally advanced flap for the treatment of buccal gingival recessions with and without enamel matrix derivative. A split-mouth study. *J Periodontol* 2000;71:1693-1698.

19. Hagewald S, Spahr A, Rompola E, Haller B, Heijl L, Bernimoulin JP. Comparative study of Emdogain and coronally advanced flap technique in the treatment of human gingival recessions. A prospective controlled clinical study. *J Clin Periodontol* 2002;29:35-41.

20. Aroca S, Keglevich T, Nikolidakis D, et al. Treatment of class III multiple gingival recessions: a randomized-clinical trial. *J Clin Periodontol* 2010;37:88-97.

21. Tavelli L, Barootchi S, Cairo F, Rasperini G, Shedden K, Wang HL. The Effect of Time on Root Coverage Outcomes: A Network Meta-Analysis. *J Dent Res* 2019.

22. McGuire MK, Scheyer ET, Schupbach P. A Prospective, Case-Controlled Study Evaluating the Use of Enamel Matrix Derivative on Human Buccal Recession Defects: A Human Histologic Examination. *J Periodontol* 2016;87:645-653.

23. Sangiorgio JPM, Neves F, Rocha Dos Santos M, et al. Xenogenous Collagen Matrix and/or Enamel Matrix Derivative for Treatment of Localized Gingival Recessions: A Randomized Clinical Trial. Part I: Clinical Outcomes. *J Periodontol* 2017;88:1309-1318.

24. Alves LB, Costa PP, Scombatti de Souza SL, et al. Acellular dermal matrix graft with or without enamel matrix derivative for root coverage in smokers: a randomized clinical study. *J Clin Periodontol* 2012;39:393-399.

25. Shin SH, Cueva MA, Kerns DG, Hallmon WW, Rivera-Hidalgo F, Nunn ME. A comparative study of root coverage using acellular dermal matrix with and without enamel matrix derivative. *J Periodontol* 2007;78:411-421.

26. Costa PP, Alves LB, Souza SL, et al. Root Coverage in Smokers with Acellular Dermal Matrix Graft and Enamel Matrix Derivative: A 12-Month Randomized Clinical Trial. *Int J Periodontics Restorative Dent* 2016;36:525-531.

27. Cairo F, Pagliaro U, Buti J, et al. Root coverage procedures improve patient aesthetics. A systematic review and Bayesian network meta-analysis. *J Clin Periodontol* 2016;43:965-975.

28. Rocha Dos Santos M, Sangiorgio JPM, Neves F, et al. Xenogenous Collagen Matrix and/or Enamel Matrix Derivative for Treatment of Localized Gingival Recessions: A Randomized Clinical Trial. Part II: Patient-Reported Outcomes. *J Periodontol* 2017;88:1319-1328.

29. Lynch SE, Williams RC, Polson AM, et al. A combination of platelet-derived and insulin-like growth factors enhances periodontal regeneration. *J Clin Periodontol* 1989;16:545-548.

30. Cho MI, Lin WL, Genco RJ. Platelet-derived growth factor-modulated guided tissue regenerative therapy. *J Periodontol* 1995;66:522-530.

31. Camelo M, Nevins ML, Schenk RK, Lynch SE, Nevins M. Periodontal regeneration in human Class II furcations using purified recombinant human platelet-derived growth factor-BB (rhPDGF-BB) with bone allograft. *Int J Periodontics Restorative Dent* 2003;23:213-225.

32. Nevins M, Giannobile WV, McGuire MK, et al. Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: results of a large multicenter randomized controlled trial. *J Periodontol* 2005;76:2205-2215.

33. Park JB, Matsuura M, Han KY, et al. Periodontal regeneration in class III furcation defects of beagle dogs using guided tissue regenerative therapy with platelet-derived growth factor. *J Periodontol* 1995;66:462-477.

34. Matsuda N, Lin WL, Kumar NM, Cho MI, Genco RJ. Mitogenic, chemotactic, and synthetic responses of rat periodontal ligament fibroblastic cells to polypeptide growth factors in vitro. *J Periodontol* 1992;63:515-525.

35. Dennison DK, Vallone DR, Pinero GJ, Rittman B, Caffesse RG. Differential effect of TGF-beta 1 and PDGF on proliferation of periodontal ligament cells and gingival fibroblasts. *J Periodontol* 1994;65:641-648.

36. Boyan LA, Bhargava G, Nishimura F, Orman R, Price R, Terranova VP. Mitogenic and chemotactic responses of human periodontal ligament cells to the different isoforms of platelet-derived growth factor. *J Dent Res* 1994;73:1593-1600.

37. Zadeh HH. Minimally invasive treatment of maxillary anterior gingival recession defects by vestibular incision subperiosteal tunnel access and platelet-derived growth factor BB. *Int J Periodontics Restorative Dent* 2011;31:653-660.

38. Carney CM, Rossmann JA, Kerns DG, et al. A comparative study of root defect coverage using an acellular dermal matrix with and without a recombinant human platelet-derived growth factor. *J Periodontol* 2012;83:893-901.

39. Rubins RP, Tolmie PN, Corsig KT, Kerr EN, Kim DM. Subepithelial connective tissue graft with purified rhPDGF-BB for the treatment of mandibular recession defects: a consecutive case series. *Int J Periodontics Restorative Dent* 2014;34:315-321.

40. Deshpande A, Koudale SB, Bhongade ML. A comparative evaluation of rhPDGF-BB + beta-TCP and subepithelial connective tissue graft for the treatment of multiple gingival recession defects in humans. *Int J Periodontics Restorative Dent* 2014;34:241-249.

41. McGuire MK, Scheyer ET, Snyder MB. Evaluation of recession defects treated with coronally advanced flaps and either recombinant human platelet-derived growth factor-BB plus beta-tricalcium phosphate or connective tissue: comparison of clinical parameters at 5 years. *J Periodontol* 2014;85:1361-1370.

42. Del Fabbro M, Karanxha L, Panda S, et al. Autologous platelet concentrates for treating periodontal infrabony defects. *Cochrane Database Syst Rev* 2018;11:CD011423.

43. Comert Kilic S, Gungormus M, Parlak SN. Histologic and histomorphometric assessment of sinus-floor augmentation with beta-tricalcium phosphate alone or in combination with pure-platelet-rich plasma or platelet-rich fibrin: A randomized clinical trial. *Clin Implant Dent Relat Res* 2017;19:959-967.

44. Aroca S, Keglevich T, Barbieri B, Gera I, Etienne D. Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study. *J Periodontol* 2009;80:244-252.

45. Kuka S, Ipci SD, Cakar G, Yilmaz S. Clinical evaluation of coronally advanced flap with or without platelet-rich fibrin for the treatment of multiple gingival recessions. *Clin Oral Investig* 2018;22:1551-1558.

46. Castro AB, Meschi N, Temmerman A, et al. Regenerative potential of leucocyte- and platelet-rich fibrin. Part A: intra-bony defects, furcation defects and periodontal plastic surgery. A systematic review and meta-analysis. *J Clin Periodontol* 2017;44:67-82.

47. Huang LH, Neiva RE, Soehren SE, Giannobile WV, Wang HL. The effect of platelet-rich plasma on the coronally advanced flap root coverage procedure: a pilot human trial. *J Periodontol* 2005;76:1768-1777.

48. Keceli HG, Sengun D, Berberoglu A, Karabulut E. Use of platelet gel with connective tissue grafts for root coverage: a randomized-controlled trial. *J Clin Periodontol* 2008;35:255-262.

49. Jenabian N, Motallebnejad M, Zahedi E, Sarmast ND, Angelov N. Coronally advanced flap and connective tissue graft with or without plasma rich in growth factors (PRGF) in treatment of gingival recession. *J Clin Exp Dent* 2018;10:e431-e438.

50. Larsson L, Decker AM, Nibali L, Pilipchuk SP, Berglundh T, Giannobile WV. Regenerative Medicine for Periodontal and Peri-implant Diseases. *J Dent Res* 2016;95:255-266.

51. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:e51-55.

52. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:e45-50.

53. Keceli HG, Kamak G, Erdemir EO, Evginer MS, Dolgun A. The Adjunctive Effect of Platelet-Rich Fibrin to Connective Tissue Graft in the Treatment of Buccal Recession Defects: Results of a Randomized, Parallel-Group Controlled Trial. *J Periodontol* 2015;86:1221-1230.

54. Femminella B, Iaconi MC, Di Tullio M, et al. Clinical Comparison of Platelet-Rich Fibrin and a Gelatin Sponge in the Management of Palatal Wounds After Epithelialized Free Gingival Graft Harvest: A Randomized Clinical Trial. *J Periodontol* 2016;87:103-113.

55. Ozcan M, Ucak O, Alkaya B, Keceli S, Seydaoglu G, Haytac MC. Effects of Platelet-Rich Fibrin on Palatal Wound Healing After Free Gingival Graft Harvesting: A Comparative Randomized Controlled Clinical Trial. *Int J Periodontics Restorative Dent* 2017;37:e270-e278.

56. Oncu E. The Use of Platelet-Rich Fibrin Versus Subepithelial Connective Tissue Graft in Treatment of Multiple Gingival Recessions: A Randomized Clinical Trial. *Int J Periodontics Restorative Dent* 2017;37:265-271.

57. Cairo F, Nieri M, Pagliaro U. Efficacy of periodontal plastic surgery procedures in the treatment of localized facial gingival recessions. A systematic review. *J Clin Periodontol* 2014;41 Suppl 15:S44-62.

58. Yeh YL, Kang YM, Chaibi MS, Xie JF, Graves DT. IL-1 and transforming growth factor-beta inhibit platelet-derived growth factor-AA binding to osteoblastic cells by reducing platelet-derived growth factor-alpha receptor expression. *J Immunol* 1993;150:5625-5632.

59. Oates TW, Rouse CA, Cochran DL. Mitogenic effects of growth factors on human periodontal ligament cells in vitro. *J Periodontol* 1993;64:142-148.

60. Nagayasu-Tanaka T, Anzai J, Takaki S, et al. Action Mechanism of Fibroblast Growth Factor-2 (FGF-2) in the Promotion of Periodontal Regeneration in Beagle Dogs. *PLoS One* 2015;10:e0131870.

61. Shujaa Addin A, Akizuki T, Hoshi S, et al. Biodegradable gelatin/beta-tricalcium phosphate sponges incorporating recombinant human fibroblast growth factor-2 for treatment of recession-type defects: A split-mouth study in dogs. *J Periodontal Res* 2017;52:863-871.

62. Murakami S. Periodontal tissue regeneration by signaling molecule(s): what role does basic fibroblast growth factor (FGF-2) have in periodontal therapy? *Periodontol 2000* 2011;56:188-208.

63. Cochran DL, Oh TJ, Mills MP, et al. A Randomized Clinical Trial Evaluating rh-FGF-2/β-TCP in Periodontal Defects. J Dent Res. 2016; 95:523-30.

64. Kitamura M, Akamatsu M, Kawanami M, et al. Randomized Placebo-Controlled and Controlled Non-Inferiority Phase III Trials Comparing Trafermin, a Recombinant Human Fibroblast Growth Factor 2, and Enamel Matrix Derivative in Periodontal Regeneration in Intrabony Defects. J Bone Miner Res. 2016; 31:806-14.

65. Kitamura M, Akamatsu M, Machigashira M, et al. FGF-2 stimulates periodontal regeneration: results of a multi-center randomized clinical trial. *J Dent Res* 2011;90:35-40.

66. Ishii Y, Fujita T, Okubo N, Ota M, Yamada S, Saito A. Effect of basic fibroblast growth factor (FGF-2) in combination with beta tricalcium phosphate on root coverage in dog. *Acta Odontol Scand* 2013;71:325-332.

67. Sallum EA, Casati MZ, Caffesse RG, Funis LP, Nociti Junior FH, Sallum AW. Coronally positioned flap with or without enamel matrix protein derivative for the treatment of gingival recessions. *Am J Dent* 2003;16:287-291.

68. Cha JK, Sun YK, Lee JS, Choi SH, Jung UW. Root coverage using porcine collagen matrix with fibroblast growth factor-2: a pilot study in dogs. *J Clin Periodontol* 2017;44:96-103.

69. Simion M, Rocchietta I, Fontana F, Dellavia C. Evaluation of a resorbable collagen matrix infused with rhPDGF-BB in peri-implant soft tissue augmentation: a preliminary report with 3.5 years of observation. *Int J Periodontics Restorative Dent* 2012;32:273-282.

70. Ebell MH, Siwek J, Weiss BD, et al. Strength of recommendation taxonomy (SORT): a patient-centered approach to grading evidence in the medical literature. *Am Fam Physician* 2004;69:548-556.

**Figure Legend**

**Figure 1.** Root coverage procedure with connective tissue graft + EMD. **A)** Baseline defect; **B)** Split-full-split flap elevation; **C)** After root conditioning with 24% EDTA. EMD was applied to the root surface; **D)** CTG sutured over the EMD and the root surfaces; **E)** Flap closure; **F)** 1-year outcomes showing complete root coverage.

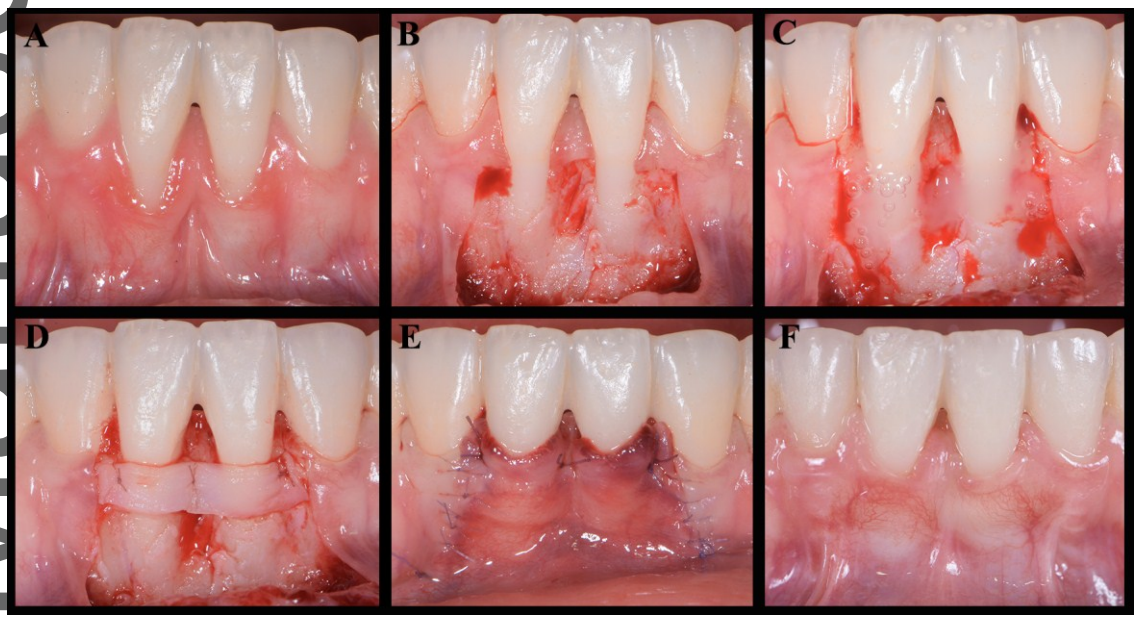


**Figure 2.** Clinical and histological efficacy of EMD in the regeneration of recession defects. **A)** Test gingival recession defect 8 weeks after the creation of the recession; **B)** Histologic marker being placed into root surface at the position of the free gingival margin after root planing and application of EDTA; **C)** Full-thickness flap creating recipient bed showing notch at the gingival margin level and relationship to the alveolar bone crest; **D)** Insertion of histologic notch into root surface at the level of the alveolar bone crest; **E)** Application of EMD over root surface; **F)** Healing at 9 months showing complete root coverage; **G)** Low-power ground section demonstrating both notches, newly formed bone (NB), and old and new cementum (OC and NC, respectively); **H)** Ground section showing new bone (NB) separated from old cementum (OC) by newly-formed periodontal ligament (PL); **I)** New bone (NB) covering previously exposed root surface. Root surface is covered by both old (OC) and new cementum (NC); **J)** Continuing down the root surface, new bone (NB), periodontal ligament (PL) and cementum (NC) can be seen covering the notch at the original gingival margin level; **K)** Section under polarized light showing newly formed periodontal ligament fibers (PL) between newly formed bone (NB) and old cementum (OC). (Adapted with permission from Journal of Periodontology [22]).

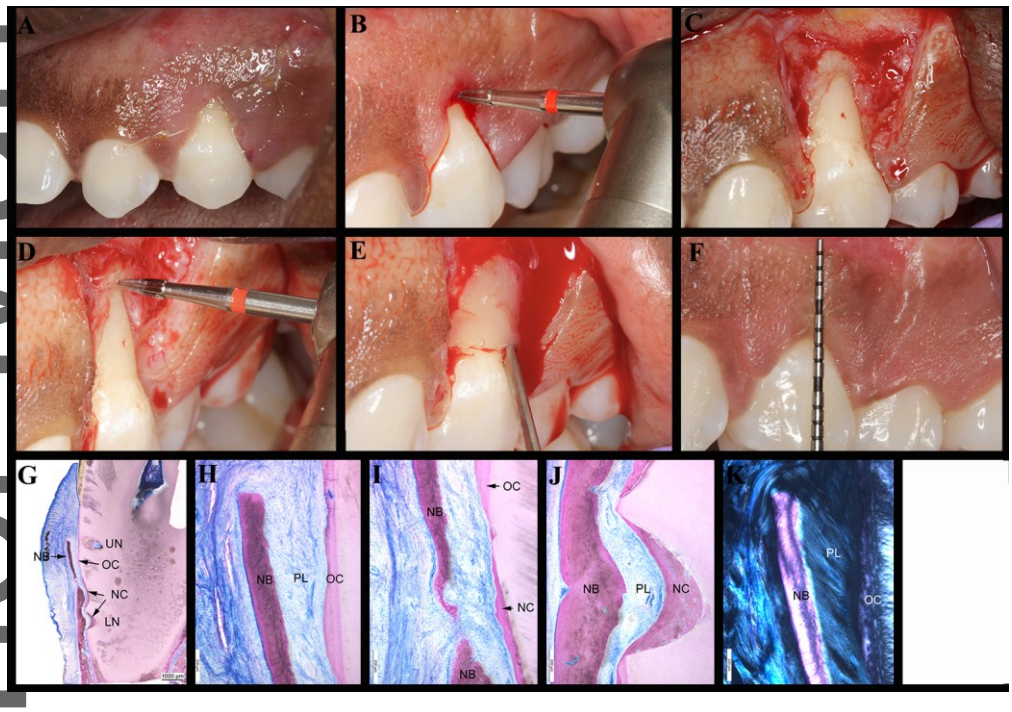
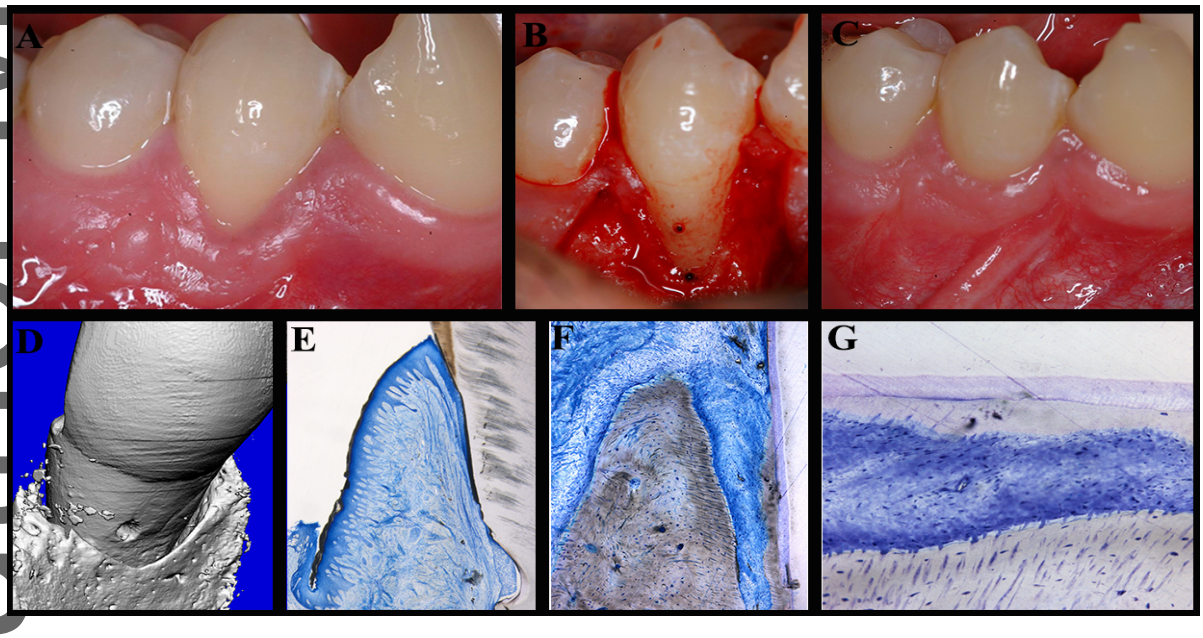


**Figure 3.** Clinical and histological efficacy of rhPDGF-BB in the regeneration of recession defects. **A)** Clinical photograph of recession defect 2 months after its surgical creation. Osseous and gingival reference notches were placed at the time of surgical correction of the recession defect; **B)** In each case, the osseous crest was placed approximately 3 mm apical to the pre-corrected gingival margin; **C)** Complete root coverage is maintained 9 months after correction of the recession defect with rhPDGF-BB + β-TCP; **D)** Nine months after grafting with rhPDGF-BB + β-TCP, micro-CT reveals coronal bone regeneration superior to the osseous notch; **E-G)** Ground sections demonstrated robust coronal bone regeneration and newly formed cementum 9 months after grafting with rhPDGF-BB + β-TCP; **G)** Higher-power ground section revealing regeneration of all tissues of missing periodontium. (Adapted with permission from Journal of Periodontology [10]).



**Tables**

**Table 1.** Summary of the biologics-based technologies for application in root coverage procedures

| Biologic agent | Origin | Properties | Carrier Matrix | Level of Evidence (SORT) | Reference(s) |
|---|---|---|---|---|---|
| Enamel matrix derivative (EMD) | Porcine fetal tooth | • Stimulating cementogenesis<br>• Enhancing proliferation, differentiation and migration of PDL cells and osteoblasts<br>• Enhancing blood vessels formation<br>• Promoting growth factor expression | Can be used alone (gel) or with an absorbable collagen sponge | A | Rasperini et al. 2011, McGuire et al. 2016, Sangiorgio et al. 2017 [6, 22, 23] |
| Recombinant human Platelet-derived growth factor (rhPDGF-BB) | Molecularly cloned from human PDGF-B gene | • Promoting bone, cementum and PDL regeneration<br>• Enhancing proliferation and chemotaxis of PDL and alveolar bone cells | Can be used alone (gel) or with different scaffolds (β-TCP, DFDBA, FDBA) | B | McGuire & Scheyer 2006, McGuire et al. 2009, Simion et al. 2012 [9, 10, 69] |
| Platelet-rich fibrin (PRF) | Centrifugation of the patient's own blood without | • Releasing of low concentrations of growth factors | Has been used alone (as a membrane) or in combination | B | Keceli et al. 2008, Kuka et al. 2018 [45, 48] |

| | | | | | |
|---|---|---|---|---|---|
| | the addition of anticoagulants | (including PDGF, VEGF, TGF β-1 and IGF-1) | with soft tissue grafts | | |
| Fibroblast growth factor-2 (FGF-2) | Molecularly cloned from human FGF-2 gene | • Enhancing angiogenic and osteogenic activity<br>• Stimulating the proliferation and migration of PDL cells | Can be used alone, with an absorbable sponge, a collagen matrix or β-TCP | C | Ishii et al. 2013, Cha et al. 2017 [66, 68] |

**Legend.** SORT: Strength-of-Recommendation Taxonomy. SORT A: consistent, good-quality patient-oriented evidence; SORT B: inconsistent or limited-quality patient-oriented evidence; SORT-C: consensus, disease-oriented evidence, usual practice, expert opinion or case series for studies of diagnosis, treatment, prevention, or screening [70]