Biologic Agents to Promote Periodontal Regeneration and Bone Augmentation

Jill D. Bashutski* and Hom-Lay Wang*

Periodontal regeneration is preferred over tissue repair and is accomplished through the exclusion of epithelial tissues, which allows cementum, bone, and connective tissue to repopulate the wound. Recently, biologic materials have emerged as adjuncts to aid in regeneration by augmenting the events of wound healing in the area. A review of biologic agents was conducted using the following MeSH terms: guided tissue regeneration, intercellular signaling peptides and proteins, and biologic factors.

Enamel matrix derivative (EMD), platelet-derived growth factor (PDGF), platelet-rich plasma, bone morphogenetic proteins (BMPs), fibroblast growth factor (FGF), and parathyroid hormone (PTH) have all shown promise in promoting hard- or soft-tissue regeneration. No biologic agent is ideal for all clinical situations so the clinician must evaluate each situation to identify the best indication for its usage. Currently, EMD and PDGF have Food and Drug Administration approval for periodontal regeneration, whereas BMP-2 is approved for bone augmentation. FGF and PTH do not have Food and Drug Administration approval for periodontal applications and so their clinical usage is not indicated. *Clin Adv Periodontics* 2011;1:80-87.

Key Words: Guided tissue regeneration, periodontal; platelet-derived growth factor.

Background

Newer approaches to periodontal therapy include regenerative procedures that aim to restore lost periodontal ligament, bone, cementum, and connective tissue. When the goal is to restore periodontal tissues around teeth, this is referred to as guided tissue regeneration (GTR). Guided bone regeneration (GBR) is also used to provide bone augmentation in edentulous areas, usually before implant placement. Regenerative therapy has an advantage over traditional surgical approaches in that the original tissues are reestablished, not replaced through a reparative process. Regenerative procedures typically use barrier membranes and bone grafting materials to encourage the growth of key periodontal tissues but exclude unwanted cell types, such as epithelial cells and connective tissue fibroblasts.[1-4] Although regenerative therapies have great potential, they remain unpredictable in their ability to produce acceptable outcomes in all situations consistently. Laurell et al.[5] showed that, when a bone graft alone was placed in infrabony periodontal defects, limited pocket reduction was achieved but clinical attachment level (CAL) gain and bone fill were significantly improved, averaging 2.1 mm. When GTR with both bone graft and a membrane were used, GTR resulted in significant pocket reduction, CAL gain of 4.2 mm, and bone fill averaging 3.2 mm.[5] The predictability of GTR, however, has been questioned. Eikholz et al.[6] treated 50 infrabony defects with GTR. After 5 years, 47 of the defects still showed evidence of vertical attachment gain, but three defects had a net loss of up to 2.2 mm. In a systematic review of the literature, Murphy and Gunsolley[7] found some heterogeneity in the outcome of GTR and concluded that it was difficult to assess the efficacy of periodontal treatment using physical barriers because no studies were performed that measured tooth retention for >5 years. Consequently, a need exists to augment current GTR techniques to improve outcomes and predictability.

To help promote periodontal regeneration and healing, the local application of biologic modifiers such as growth factors has been investigated. These agents act by augmenting the wound healing process through anabolic bone formation, angiogenesis, cementogenesis, osteoblast differentiation, mitosis, chemotaxis, and other processes that improve the healing environment. Biologic modifiers, including enamel matrix derivative (EMD), platelet-derived growth factor (PDGF), bone morphogenetic protein (BMP), platelet-rich plasma (PRP), fibroblast growth factor (FGF), and parathyroid hormone (PTH), have all shown promise in enhancing regeneration.[8-12] When choosing which agent to use, it is important to consider several factors, including the evidence supporting its use as well as the specific clinical situation. An electronic literature search of biologic agents was conducted in the PubMed (MEDLINE) database using the following MeSH terms: guided tissue regeneration, intercellular signaling peptides and proteins, and biologic factors. Other key search terms included bone morphogenic proteins, parathyroid hormone, platelet-derived growth factor, enamel matrix derivative, fibroblast growth factor, platelet-rich plasma, and related terms. Animal and human studies published in the English language up to January 2011 were reviewed. This paper presents an overview of the different

* *Department of Periodontics and Oral Medicine, School of Dentistry, University of Michigan, Ann Arbor, MI.*

Submitted April 24, 2011; accepted for publication June 8, 2011

doi: 10.1902/cap.2011.110044

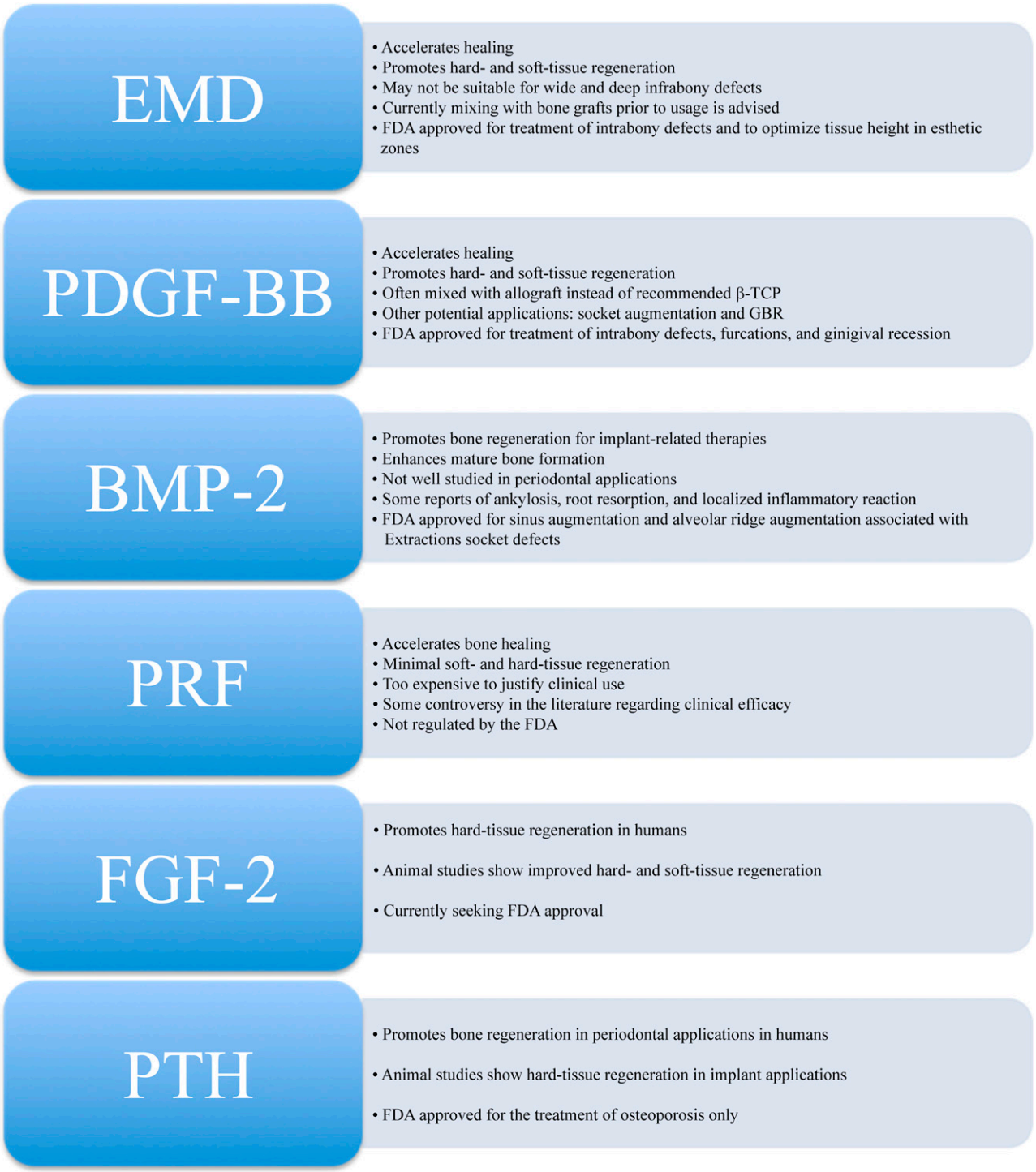

FIGURE 1 Summary of the mechanism of action, indications, and FDA approval status of common biologic agents to promote periodontal regeneration. TCP = tricalcium phosphate.

biologic agents that can be used as adjuncts for GTR and GBR as well as a decision model for deciding on the best agent for a given clinical scenario (Fig. 1).

Enamel Matrix Derivative

Physiologically, enamel matrix proteins are secreted by Hertwig's epithelial root sheath during root development and serve to promote cementogenesis.[13] A recent review by Bosshardt[14] suggests that there is strong evidence for enamel matrix proteins to support wound healing and new periodontal tissue formation, but that the biologic mechanism by which this occurs is still unclear. A commercially available form of EMD† is available and is derived from porcine tooth buds, and is approved by the Food and Drug Administration (FDA) for the treatment of moderate or severe periodontitis as well as to optimize tissue

† Emdogain, Straumann, Basel, Sweden.

height in esthetic zones. It is only approved for intrabony defects, not furcation areas, although there is evidence that EMD is effective in treating grade 2 furcations.[15,16] EMD is perhaps one of the most extensively studied biologic agents, and Table 1 summarizes some key studies evaluating the use of EMD to promote regeneration.

Platelet-Derived Growth Factor

PDGF-BB is a growth factor involved in wound healing that stimulates the regenerative potential of periodontal tissues, including bone, cementum, and periodontal ligament.[17] The commercially available form‡ is FDA approved for the treatment of intrabony defects, furcations, and gingival recession associated with periodontal defects. It is specifically contraindicated in areas of acute infection, although the use of any biomaterial is contraindicated in infected areas. In addition, it should not be used in areas in which surgical soft-tissue coverage is necessary but unobtainable and in areas in which bone grafting is not advisable. Several clinical and animal trials support its use, which are summarized in Table 2.

Bone Morphogenetic Proteins (BMP-2)

BMPs have an anabolic effect on periodontal tissues through stimulation of osteoblastic differentiation in human periodontal ligament cells.[6] Some preliminary evidence suggested that BMP-2 could induce periodontal regeneration, but some adverse healing events such as ankylosis and root resorption were also reported.[18,19] The commercially available form§ is FDA approved as an alternative to autogenous bone grafting for sinus augmentations and localized alveolar ridge augmentations for defects associated with extraction sockets. Table 3 summarizes the available evidence on the efficacy of BMP-2 as a biologic agent.

Platelet-Rich Fibrin Glue

Platelet-rich fibrin glue (PRFG) consists of uncoagulated blood that is centrifuged using a commercially available system to produce a layer of increased concentrations of platelets and leukocytes within a fibrin scaffold.[20] Platelet-rich fibrin (PRF) contains high amounts of bioactive growth factors to enhance wound healing through increased chemotaxis, proliferation, differentiation, and angiogenesis.[21] PRP was extensively studied in the past, but PRF is now regarded as having more regenerative potential. PRF is not regulated by the FDA and so there are no specific approvals for this agent. Table 4 provides information regarding studies evaluating these regenerative approaches.

Fibroblast Growth Factor

FGF-2 stimulates angiogenesis and mitosis of mesenchymal cells and thus may encourage periodontal regeneration.[17] Several animal studies support its role as a regenerative agent,[22-25] and one multicenter clinical trial showed improved bone height when using it as an adjunct to periodontal regeneration.[26] It is not commercially available and does not have FDA approval. Table 5 summarizes the animal studies and one multicenter human clinical trial evaluating FGF-2.

Parathyroid Hormone

PTH is an endogenous hormone with both catabolic and anabolic properties in bone, depending on the concentration and dosing regimen. Animal studies support its ability to promote bone formation in the oral cavity for both periodontal and implant applications.[27-30] One randomized human clinical trial also showed that a commercially available form of PTH‖ promoted periodontal regeneration in infrabony defects.[12] PTH is FDA approved only for the treatment of osteoporosis. Table 6 summarizes the available evidence.

Discussion

In deciding on which agent to use, it is important to consider the evidence for a given agent as well as the clinical scenario. EMD has the largest body of clinical evidence supporting its use, with many studies showing successful results for up to 10 years.[31-34] Indications for using EMD are wide, and its use in improving hard- and soft-tissue regeneration is well supported. It may not be the ideal choice for use in treating deep and wide defects, because one study reported that EMD was not beneficial in this situation.[35] Furthermore, it is not FDA approved for the treatment of furcation areas.

PDGF-BB is supported by one of the largest prospective, randomized, triple-masked, and controlled clinical trials to assess periodontal regeneration.[9] It is indicated for enhancing both hard- and soft-tissue regeneration and is associated with a high degree of improvement in infrabony defect resolution.[36,37] Based on the studies available to date, both EMD and PDGF-BB appear to have minimal safety concerns. In addition, both show evidence that they accelerate early healing events, so these may be an ideal choice when faster healing is desirable (e.g., medically compromised patients).

In terms of available evidence, some studies[38,39] failed to support the ability of PRP to promote hard-tissue regeneration, although there appear to be some benefits to improving soft-tissue parameters and accelerating bone maturation. There is a lack of consensus on the efficacy of PRP, with some studies showing improved outcomes and others not (see Table 1). This could be attributable to the wide variation of growth factors in PRP based on differences in procurement technique and patient individuality.[40] Once the proper equipment is acquired, PRP requires little cost to operate because the patient's blood is used as the regenerative agent. The disadvantage of using this technique is that the concentration of the growth factors within PRP is highly unpredictable.[40]

BMP-2 has some human trials with long-term follow-up supporting its use in hard-tissue augmentation applications.[41,42] However, no human studies are available regarding its use in periodontal applications. In addition, there are

‡ GEM 21S, BioMimetic Pharmaceuticals, Franklin, TN.
§ INFUSE Bone Graft, Medtronic, Memphis, TN.
‖ Forteo, Eli Lilly, Indianapolis, IN.

TABLE 1 EMD

Study	Study Design	Materials and Methods	Conclusions
Grusovin and Esposito, 2009[35]	Human clinical trial	30 patients with infrabony defects; EMD versus placebo	EMD provided no additional benefits in the treatment of deep and wide infrabony defects
Sculean et al., 2008[34]	Human clinical trial	38 patients; EMD versus GTR versus EMD plus GTR versus OFD	EMD, GTR, and EMD plus GTR had significantly greater improvements than OFD at 10 years
Crea et al., 2008[31]	Human clinical trial	40 patients with infrabony defects; EMD or GTR using non-resorbable membrane	EMD resulted in significantly improved clinical and radiographic outcomes for ≤36 months
Sculean et al., 2007[45]	Human clinical trial	25 patients with infrabony defects; EMD or EMD with bioactive glass	Similar and successful results obtained between the groups for ≤4 years
Francetti et al., 2005[32]	Multicenter human clinical trial	195 intrabony defects treated with SPP flap with EMD	EMD improved the rate and degree of regeneration
Heijl et al., 1997[11]	Human clinical trial	33 patients with split-mouth design; MWF versus MWF and EMD	MWF and EMD had significantly greater radiographic bone fill and CAL gain

MWF = modified Widman flap; OFD = open flap debridement; SPP = simplified papilla preservation flap.

TABLE 2 PDGF-BB

Study	Study Design	Materials and Methods	Conclusions
Nevins et al., 2005[9]	Multicenter human clinical trial	180 patients with an infrabony defect; β-TCP plus 0.3 mg/mL rhPDGF-BB versus β-TCP plus 1.0 mg/mL rhPDGF-BB versus β-TCP	0.3 mg/mL rhPDGF-BB resulted in greater linear bone gain and defect fill at 6 months as well as faster CAL gain than controls
Howell et al.,1997[36]	Human clinical trial	38 patients, split-mouth design; flap surgery versus low-dose or high-dose PDGF-BB/IGF-I	High-dose PDGF/IGF-I resulted in significant bone regeneration
Lynch et al., 1991[37]	Animal study (dogs)	13 dogs with naturally occurring periodontitis, split-mouth study; PDGF-B/IGF-I versus carrier	PDGF-B/IGF-I resulted in significantly more new bone, cementum, and PDL formation

IGF = insulin growth factor; PDL = periodontal ligament; rh = recombinant human; TCP = tricalcium phosphate.

TABLE 3 BMP-2

Study	Study Design	Materials and Methods	Conclusions
Triplett et al., 2009[42]	Human clinical trial	160 patients; rhBMP-2 versus autograft for sinus augmentation	79% success rates in rhBMP-2 group with comparable outcomes to autograft
Jung et al., 2003[10]	Human clinical trial	11 patients requiring implant placement and GBR, split-mouth study; xenograft/membrane versus xenograft/membrane/BMP-2	BMP-2 resulted in more mature lamellar bone and increased graft-to-bone contact at 6 months
Jung et al., 2009[41]	Human clinical trial	Long-term follow-up (3 and 5 years) of implants from previous 2003 study	No significant differences observed between groups (100% success rate in both)
Wikesjö et al., 2004[46]	Animal study (dogs)	Bilateral surgically created peri-implant defects; rhBMP-2 versus placebo, both received ePTFE membrane	rhBMP-2 enhanced GBR for both smooth and rough surface implants
Saito et al., 2003[47]	Animal study (dogs)	72 surgically created periodontal defects in four dogs; rhBMP-2 versus rhBMP-2 with spacer membrane versus placebo	rhBMP-2 had greatest bone formation and CT attachment but also ankylosis; rhBMP-2 and spacer membrane minimized ankylosis

CT = connective tissue; ePTFE = expanded polytetrafluoroethylene; rh = recombinant human.

TABLE 4 PRF and PRP

Study	Study Design	Materials and Methods	Conclusions
Simon et al., 2009[48]	Animal study (dogs)	Four dogs with extracted teeth; PRF versus PRF plus membrane versus DFDBA plus membrane versus control	Faster healing in both PRF and PRF plus membrane groups but graft particles noted in coronal areas
Mazor et al., 2009[49]	Human case series	25 sinus elevations in 20 patients with PRF	No implant failures; histology showed well-organized and vital bone
Camargo et al., 2009[50]	Human clinical trial	23 patients with infrabony defects, split-mouth study; BPBM/GTR/PRP versus BPBM/GTR	PRP failed to provide additional benefits as assessed using surgical reentry at 6 months
Dori et al., 2008[51]	Human clinical trial	EMD plus PRP plus NBM versus PRP plus NBM	PRP failed to provide additional benefits
Piemontese et al., 2008[38]	Human clinical trial	60 intrabony defects; PRP plus DFDBA versus DFDBA	PRP resulted in greater PD reductions and CAL gains at 12 months; no difference in hard-tissue outcomes
You et al., 2007[52]	Animal study (dogs)	Six dogs with three implants each, surgically created peri-implant defect; autogenous bone plus PRP or PRFG	Greater BIC with PRFG

BIC = bone-to-implant contact; BPBM = bovine porous bone mineral; DFDBA = demineralized freeze-dried bone allograft; NBM = natural bone mineral; PD = probing depth.

TABLE 5 FGF-2

Study	Study Design	Materials and Methods	Conclusions
Kitamura et al., 2008[26]	Multicenter human clinical trial	74 patients with infrabony defects; placebo versus 0.03% FGF-2 versus 0.1% FGF-2 versus 0.3% FGF-2	No statistically significant differences between FGF-2 groups and control, except for greater increase in alveolar bone height between 0.3% FGF-2 and placebo at 36 weeks
Sato et al., 2004[24]	Animal study (dogs)	Surgically created defects; 0.1, 1, or 5 μg basic FGF in collagen gel versus carrier alone	1 μg basic FGF resulted in PDL formation and newly synthesized cementum at 8 weeks
Takayama et al., 2001[25]	Animal study (primates)	32 surgically created Class II furcation defects in four primates; 0.1 or 0.4% FGF-2 versus placebo	FGF-2 had significantly greater bone regeneration and was dose dependent
Hosokawa et al., 2000[53]	Animal study (dogs)	FGF-2 versus placebo in surgically created defects	FGF-2 areas had significantly more bone regeneration

PDL = periodontal ligament.

TABLE 6 PTH

Study	Study Design	Materials and Methods	Conclusions
Bashutski et al., 2010[12]	Human clinical trial	40 patients with infrabony defects; placebo versus 20 mg PTH 1 to 34 daily for 6 weeks in conjunction with periodontal surgery	PTH had significantly greater infrabony defect resolution, CAL gain, and PD reduction
Jung et al., 2007[29]	Animal study (rabbits)	Surgically created defects with titanium cylinders filled with PEG/HA/TCP versus PEG/PTH versus PEG/PTH/HA/TCP	PTH significantly increased newly formed bone in calvarial defects
Jung et al., 2007[28]	Animal study (foxhounds)	48 implants in mandible with surgically created defects; PEG/PTH versus PEG versus autogenous bone versus control	PTH group achieved greater bone regeneration
Barros et al., 2003[27]	Animal study (rodents)	Ligature-induced periodontitis model; PTH versus control	PTH protected against periodontitis-associated bone loss
Miller et al., 1997[30]	Animal study (rodents)	Mandibles of ovariectomized rats treated with PTH versus sham controls	Bone formation was greater in PTH group

HA/TCP = hydroxyapatite/tricalcium phosphate; PD = probing depth; PEG = polyethylene glycol matrix.

some reports that BMP-2 stimulates root resorption and ankylosis, so it may be best used in site augmentation and implant applications.[18] Localized inflammation in the early stage of healing have also been associated with this agent,[42,43] warranting additional study to understand the mechanism behind this occurrence.

Several animal studies support the role of FGF-2 in promoting bone and cementum formation.[23-25] In addition, one multicenter human clinical trial supports its use in improving bone regeneration.[26] Currently, FGF-2 is undergoing multicenter clinical trials to seek FDA approval.

Finally, it is also important to weigh the cost/benefit ratio because many of these biologic adjuncts have costs associated with their use. Thus, they are ideally suited to more complex and challenging cases in which outcomes are less predictable using traditional approaches.

A recent review[44] evaluating bioactive agents found that EMD (alone or with a bone graft) has positive long-term results in treating intrabony defects, with the adjunctive use of a bone graft conferring additional benefits. Intraosseous defects could also be effectively treated with PDGF-BB and peptide P-15 combined with a bone graft. In this review,[44] PRP had variable success. In addition, no bioactive agent had sufficient evidence to support its use in furcation defects.

Conclusions

Although all biologic agents have shown promise as adjuncts to regenerative procedures, certain products may be better suited to a given clinical situation. Thus, the clinician must carefully evaluate all aspects of the case before making an informed decision as to which biologic agent may be best used. ■

Acknowledgments

This study is partially supported by the University of Michigan Periodontal Graduate Student Research Fund. The authors report no conflicts of interest related to this study.

CORRESPONDENCE:
Dr. Jill Bashutski, Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, 1011 N. University Ave., Ann Arbor, MI 48109-1078. E-mail: jillbash@umich.edu.

References

1. Melcher AH. On the repair potential of periodontal tissues. *J Periodontol* 1976;47:256-260.
2. Mellonig JT, Nevins M, Sanchez R. Evaluation of a bioabsorbable physical barrier for guided bone regeneration. Part II. Material and a bone replacement graft. *Int J Periodontics Restorative Dent* 1998;18: 129-137.
3. Schwartz Z, Mellonig JT, Carnes DL Jr., et al. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation. *J Periodontol* 1996;67:918-926.
4. Gouldin AG, Fayad S, Mellonig JT. Evaluation of guided tissue regeneration in interproximal defects. (II). Membrane and bone versus membrane alone. *J Clin Periodontol* 1996;23:485-491.
5. Laurell L, Gottlow J, Zybutz M, Persson R. Treatment of intrabony defects by different surgical procedures. A literature review. *J Periodontol* 1998;69:303-313.
6. Eickholz P, Krigar DM, Kim TS, Reitmeir P, Rawlinson A. Stability of clinical and radiographic results after guided tissue regeneration in infrabony defects. *J Periodontol* 2007;78:37-46.
7. Murphy KG, Gunsolley JC. Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects. A systematic review. *Ann Periodontol* 2003;8:266-302.
8. Giannobile WV, Somerman MJ. Growth and amelogenin-like factors in periodontal wound healing. A systematic review. *Ann Periodontol* 2003; 8:193-204.
9. 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.
10. Jung RE, Glauser R, Schärer P, Hämmerle CH, Sailer HF, Weber FE. Effect of rhBMP-2 on guided bone regeneration in humans. *Clin Oral Implants Res* 2003;14:556-568.
11. Heijl L, Heden G, Svärdström G, Ostgren A. Enamel matrix derivative (EMDOGAIN) in the treatment of intrabony periodontal defects. *J Clin Periodontol* 1997;24:705-714.
12. Bashutski JD, Eber RM, Kinney JS, et al. Teriparatide and osseous regeneration in the oral cavity. *N Engl J Med* 2010;363:2396-2405.
13. Lindskog S, Hammarström L. Formation of intermediate cementum. III. ^{3}H-tryptophan and ^{3}H-proline uptake into the epithelial root sheath of Hertwig in vitro. *J Craniofac Genet Dev Biol* 1982;2:171-177.
14. Bosshardt DD. Biological mediators and periodontal regeneration: A review of enamel matrix proteins at the cellular and molecular levels. *J Clin Periodontol* 2008; 35(Suppl. 8):87-105.
15. Casarin RC, Ribeiro Edel P, Nociti FH Jr., et al. Enamel matrix derivative proteins for the treatment of proximal class II furcation involvements: A prospective 24-month randomized clinical trial. *J Clin Periodontol* 2010;37:1100-1109.
16. Hoffmann T, Richter S, Meyle J, et al. A randomized clinical multicentre trial comparing enamel matrix derivative and membrane treatment of buccal class II furcation involvement in mandibular molars. Part III. Patient factors and treatment outcome. *J Clin Periodontol* 2006;33:575-583.
17. Dereka XE, Markopoulou CE, Vrotsos IA. Role of growth factors on periodontal repair. *Growth Factors* 2006;24:260-267.
18. Sorensen RG, Wikesjö UM, Kinoshita A, Wozney JM. Periodontal repair in dogs: Evaluation of a bioresorbable calcium phosphate cement (Ceredex) as a carrier for rhBMP-2. *J Clin Periodontol* 2004;31:796-804.
19. Takahashi D, Odajima T, Morita M, Kawanami M, Kato H. Formation and resolution of ankylosis under application of recombinant human bone morphogenetic protein-2 (rhBMP-2) to class III furcation defects in cats. *J Periodontal Res* 2005;40:299-305.
20. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I. Technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:e37-e44.
21. Cochran DL, Wozney JM. Biological mediators for periodontal regeneration. *Periodontol 2000* 1999;19:40-58.
22. Hurley MM, Okada Y, Xiao L, et al. Impaired bone anabolic response to parathyroid hormone in Fgf2−/− and Fgf2+/− mice. *Biochem Biophys Res Commun* 2006;341:989-994.
23. Oi Y, Ota M, Yamamoto S, Shibukawa Y, Yamada S. Beta-tricalcium phosphate and basic fibroblast growth factor combination enhances periodontal regeneration in intrabony defects in dogs. *Dent Mater J* 2009;28:162-169.
24. Sato Y, Kikuchi M, Ohata N, Tamura M, Kuboki Y. Enhanced cementum formation in experimentally induced cementum defects of the root surface with the application of recombinant basic fibroblast growth factor in collagen gel in vivo. *J Periodontol* 2004;75:243-248.
25. Takayama S, Murakami S, Shimabukuro Y, Kitamura M, Okada H. Periodontal regeneration by FGF-2 (bFGF) in primate models. *J Dent Res* 2001;80:2075-2079.
26. Kitamura M, Nakashima K, Kowashi Y, et al. Periodontal tissue regeneration using fibroblast growth factor-2: Randomized controlled phase II clinical trial. *PLoS One* 2008;3:e2611.
27. Barros SP, Silva MA, Somerman MJ, Nociti FH Jr. Parathyroid hormone protects against periodontitis-associated bone loss. *J Dent Res* 2003;82: 791-795.
28. Jung RE, Cochran DL, Domken O, et al. The effect of matrix bound parathyroid hormone on bone regeneration. *Clin Oral Implants Res* 2007;18:319-325.
29. Jung RE, Hämmerle CH, Kokovic V, Weber FE. Bone regeneration using a synthetic matrix containing a parathyroid hormone peptide combined with a grafting material. *Int J Oral Maxillofac Implants* 2007;22:258-266.
30. Miller SC, Hunziker J, Mecham M, Wronski TJ. Intermittent parathyroid hormone administration stimulates bone formation in the mandibles of aged ovariectomized rats. *J Dent Res* 1997;76:1471-1476.
31. Crea A, Dassatti L, Hoffmann O, Zafiropoulos GG, Deli G. Treatment of intrabony defects using guided tissue regeneration or enamel matrix derivative: A 3-year prospective randomized clinical study. *J Periodontol* 2008;79:2281-2289.
32. Francetti L, Trombelli L, Lombardo G, et al. Evaluation of efficacy of enamel matrix derivative in the treatment of intrabony defects: A 24-month multicenter study. *Int J Periodontics Restorative Dent* 2005;25: 461-473.
33. Jepsen S, Topoll H, Rengers H, et al. Clinical outcomes after treatment of intra-bony defects with an EMD/synthetic bone graft or EMD alone: A multicentre randomized-controlled clinical trial. *J Clin Periodontol* 2008;35:420-428.
34. Sculean A, Kiss A, Miliauskaite A, Schwarz F, Arweiler NB, Hannig M. Ten-year results following treatment of intra-bony defects with enamel matrix proteins and guided tissue regeneration. *J Clin Periodontol* 2008; 35:817-824.
35. Grusovin MG, Esposito M. The efficacy of enamel matrix derivative (Emdogain) for the treatment of deep infrabony periodontal defects: A placebo-controlled randomised clinical trial. *Eur J Oral Implantology* 2009;2:43-54.
36. Howell TH, Fiorellini JP, Paquette DW, Offenbacher S, Giannobile WV, Lynch SE. A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. *J Periodontol* 1997;68:1186-1193.
37. Lynch SE, de Castilla GR, Williams RC, et al. The effects of short-term application of a combination of platelet-derived and insulin-like growth factors on periodontal wound healing. *J Periodontol* 1991;62:458-467.
38. Piemontese M, Aspriello SD, Rubini C, Ferrante L, Procaccini M. Treatment of periodontal intrabony defects with demineralized freeze-dried bone allograft in combination with platelet-rich plasma: A comparative clinical trial. *J Periodontol* 2008;79:802-810.
39. Sammartino G, Tia M, Gentile E, Marenzi G, Claudio PP. Platelet-rich plasma and resorbable membrane for prevention of periodontal defects after deeply impacted lower third molar extraction. *J Oral Maxillofac Surg* 2009;67:2369-2373.
40. Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts. *Clin Oral Implants Res* 2006;17:212-219.
41. Jung RE, Windisch SI, Eggenschwiler AM, Thoma DS, Weber FE, Hämmerle CH. A randomized-controlled clinical trial evaluating clinical and radiological outcomes after 3 and 5 years of dental implants placed in bone regenerated by means of GBR techniques with or without the addition of BMP-2. *Clin Oral Implants Res* 2009;20:660-666.
42. Triplett RG, Nevins M, Marx RE, et al. Pivotal, randomized, parallel evaluation of recombinant human bone morphogenetic protein-2/absorbable collagen sponge and autogenous bone graft for maxillary sinus floor augmentation. *J Oral Maxillofac Surg* 2009;67:1947-1960.
43. Boyne PJ, Lilly LC, Marx RE, et al. De novo bone induction by recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillary sinus floor augmentation. *J Oral Maxillofac Surg* 2005;63:1693-1707.
44. Trombelli L, Farina R. Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. *J Clin Periodontol* 2008; 35(Suppl. 8):117-135.
45. Sculean A, Pietruska M, Arweiler NB, Auschill TM, Nemcovsky C. Four-year results of a prospective-controlled clinical study evaluating

healing of intra-bony defects following treatment with an enamel matrix protein derivative alone or combined with a bioactive glass. *J Clin Periodontol* 2007;34:507-513.

46. Wikesjö UM, Qahash M, Thomson RC, et al. rhBMP-2 significantly enhances guided bone regeneration. *Clin Oral Implants Res* 2004;15: 194-204.
47. Saito E, Saito A, Kawanami M. Favorable healing following space creation in rhBMP-2-induced periodontal regeneration of horizontal circumferential defects in dogs with experimental periodontitis. *J Periodontol* 2003;74:1808-1815.
48. Simon BI, Zatcoff AL, Kong JJ, O'Connell SM. Clinical and histological comparison of extraction socket healing following the use of autologous platelet-rich fibrin matrix (PRFM) to ridge preservation procedures employing demineralized freeze dried bone allograft material and membrane. *Open Dent J* 2009;3:92-99.
49. Mazor Z, Horowitz RA, Del Corso M, Prasad HS, Rohrer MD, Dohan Ehrenfest DM. Sinus floor augmentation with simultaneous implant placement using Choukroun's platelet-rich fibrin as the sole grafting material: A radiologic and histologic study at 6 months. *J Periodontol* 2009;80:2056-2064.
50. Camargo PM, Lekovic V, Weinlaender M, Divnic-Resnik T, Pavlovic M, Kenney EB. A surgical reentry study on the influence of platelet-rich plasma in enhancing the regenerative effects of bovine porous bone mineral and guided tissue regeneration in the treatment of intrabony defects in humans. *J Periodontol* 2009;80:915-923.
51. Döri F, Nikolidakis D, Húszár T, Arweiler NB, Gera I, Sculean A. Effect of platelet-rich plasma on the healing of intrabony defects treated with an enamel matrix protein derivative and a natural bone mineral. *J Clin Periodontol* 2008;35:44-50.
52. You TM, Choi BH, Zhu SJ, et al. Platelet-enriched fibrin glue and platelet-rich plasma in the repair of bone defects adjacent to titanium dental implants. *Int J Oral Maxillofac Implants* 2007;22:417-422.
53. Hosokawa R, Kikuzaki K, Kimoto T, et al. Controlled local application of basic fibroblast growth factor (FGF-2) accelerates the healing of GBR. An experimental study in beagle dogs. *Clin Oral Implants Res* 2000;11: 345-353.