Future Prospects for Periodontal Bioengineering Using Growth Factors

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Polypeptide growth factors have demonstrated strong potential to repair defects associated with teeth and dental implants. Over the past two decades, intense research efforts have led to the clinical development of several growth factors or biologic agents, including bone morphogenetic proteins, platelet-derived growth factor, fibroblast growth factors, and enamel matrix proteins. Several of these growth factors are now being used clinically for a variety of applications, such as the promotion of periodontal regeneration, sinus floor augmentation, and root coverage procedures. Although clinical results have been promising and growth factors add another dimension to clinical care, optimization of growth factor targeting approaches to periodontal wounds remains a challenge. Enhancement of growth factor local application to improve bioavailability, bioactivity, and allowance of three-dimensional reconstruction of complex anatomic defects is a goal. This article will highlight developments for growth factor delivery to better stimulate the wound healing response for periodontal and bone regeneration in the maxillofacial region. *Clin Adv Periodontics* 2011;1:88-94.

Key Words: Bone morphogenetic proteins; fibroblast growth factors; platelet-derived growth factor; regeneration; regenerative medicine; tissue engineering.

Current State-of-the-Art

This special issue highlights the current status of growth factor technologies in the clinical arena. Regenerative medicine and tissue-engineering innovations have greatly advanced periodontology over the past decade.[1] Several key pivotal human clinical trials have led to the clinical application of bone morphogenetic proteins (BMPs) for localized alveolar ridge[2] and for sinus floor augmentation.[3] For platelet-derived growth factor (PDGF), it has been used for periodontal regeneration[4,5] and for the promotion of root coverage.[6] Most recently, fibroblast growth factor-2 (FGF-2) has been evaluated in a large human clinical trial for the promotion of periodontal regeneration.[7,8] As such, in this issue, Drs. Murakami et al., Misch and Wang, and Kao and Lynch highlight in greater detail not only the specific studies above but other investigations that explore expanded indications of these technologies for application in the clinical arena for oral bone and soft-tissue repair (Table 1). In this perspective, we will highlight several key areas of development that the field is undertaking to better stimulate the wound healing response for periodontal and bone regeneration in the maxillofacial region.

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Scaffolding Matrices to Enhance Growth Factor Release to Periodontal and Bone Defects

The use of scaffolding matrices to deliver growth factors to promote periodontal tissue regeneration has been an active area of research.[9] Controlled release strategies were recently characterized by Hubbell.[10] He specifically characterized controlled release strategies for tissue engineering as 1) bioactive factors mixed with matrices; 2) bioactive factors entrapped within gel matrices; 3) bioactive factors entrapped within hydrophobic microparticles; 4) bioactive factors bound to affinity sites within matrices, and 5) bioactive factors covalently bound to matrices. Of these five categories, only strategies 1, 4, and 5 present the potential for a completely integrated structural delivery because categories 2 and 3 are non-load–bearing vehicles that must be further fixed to a load-bearing structure. However, in the case of periodontal wound repair in space-making defects, the above may be an insignificant limitation. As an example of strategy 2, Lutolf et al.[11] used polyethylene glycol-based hydrogels as BMP-2 delivery vehicles in critical sized calvarial defects.

The major class of currently used BMP-2 delivery vehicles fall into category 1, "bioactive factors mixed with matrices." The best known of these is the current Food and Drug Administration–approved BMP-2 carrier bovine type I collagen sponge[12] that has been used clinically (approved or off-label) for in-spine fusion,[13-15] tibial nonunion repair,[16] and multiple craniofacial applications, including sinus lift, tooth socket repair, and cleft defects.[2,3,17]

However, collagen sponges are suboptimal delivery devices that often lead to uncontrolled bolus delivery of BMP-2, which allows diffusion of the protein into surrounding soft tissues. The presence of BMP-2 in soft tissues leads to some patient complications, including dysphagia, airway compression in cervical spine fusion, and heterotopic bone formation in the spinal canal.[18-20]

Alternative materials to collagen sponges have been proposed. Schmidmaier et al.[21,22] developed bioresorbable poly-(D, L)-lactic acid coatings for BMP-2 delivery in which the BMP-2 was mixed in a thin film of polymer. The release, as with collagen sponges, occurs when the matrix degrades and polymer diffuses from the matrix. They demonstrated significant increases in bone formation but also noted that ≈50% of growth factors using this delivery approach were eluted during the first 48 hours. This fact illustrates the drawback of this (and any) approach mixing biofactors with matrices, namely that once the matrix begins degrading, there is limited control over growth factor diffusion. As such, binding of growth factors directly to biomaterials offers significant potential (see below).

The simplest approach within Hubbell's[10] schema is to bind BMP-2 to natural or enhanced affinity binding sites on complex three-dimensional scaffold surfaces (category 4). This binding often occurs through electrostatic charge interactions between the protein and biomaterial surface. Rai et al.[23] described adsorption of BMP-2 on three-dimensional polycaprolactone (PCL) and PCL/tricalcium phosphate/fibrin composite scaffolds made by fused deposition modeling. They also found that local BMP-2 retention rates on the scaffolds ranged from ≈50% to 75% at 2 days, dropping to ≈0% to 20% by 15 days.

The last category[10] for controlled release is category 5, "bioactive factors covalently bound to matrices." This method provides the tightest binding of growth factors to substrates and is useful to retain growth factors locally as to avoid dispersion of BMPs into the soft tissues, which can lead to ectopic bone formation.[24] To date, there have not been reports related to ectopic bone formation in extraction sockets or in sinus floor elevation procedures.

In the periodontium, the use of these scaffold constructs made of a variety of formulations, such as polymeric biomaterials of polylactic glycolic acid (PLGA), PCL, or polylactic acid, have been well studied.[24] These materials have been used extensively to deliver not only growth factors but also genes and cells.[25] Additionally, a variety of ceramic biomaterials, such as hydroxyapatite, β-tricalcium phosphate, or other calcium phosphate ceramics, are being used as space-making constructs to support the three-dimensional stability of complex periodontal and peri-implant wound sites. These constructs are being used for growth factor incorporation to provide bolus, pulse, or constant slow release of growth factors. Within the polymeric constructs, microspheres or nanospheres encapsulating growth factors, antibiotics, or other anabolic agents are being used to optimize wound repair and regeneration (Fig. 1).[26] More recently, the BMP/transforming growth factor-β family member known to have a role in skeletal and joint development, growth and differentiation factor-5 (GDF-5), has demonstrated significant potential in the regeneration of periodontal ligament (PDL) and bone in vertical bone defects using PLGA and β-tricalcium phosphate carriers.[27-29] Advances in computer-aided design–computer-aided manufacturer technology over the years coupled with the expanded use of cone beam computed tomography for imaging three-dimensional structures allows for the construction of anatomically corrected scaffolds for intimate, highly accurate fitting to complex topographies of bone[30-32] and periodontal defects (Fig. 2).[33] Wikesjö et al.[34] have demonstrated that BMPs coated onto titanium porous oxide implant surfaces of dental implants allow for the release and subsequent acceleration of osseointegration in alveolar ridge defects.

TABLE 1 Growth Factors Under Investigation or Approved for Clinical Use

Growth Factor	In Vitro Evidence	Preclinical Evidence	Clinical Evidence
BMP-2	X	X	X
BMP-7	X	X	X
GDF-5	X	X	
FGF-2	X	X	X
PDGF-BB	X	X	X

Preclinical Evidence and Clinical Evidence refer to periodontal applications.

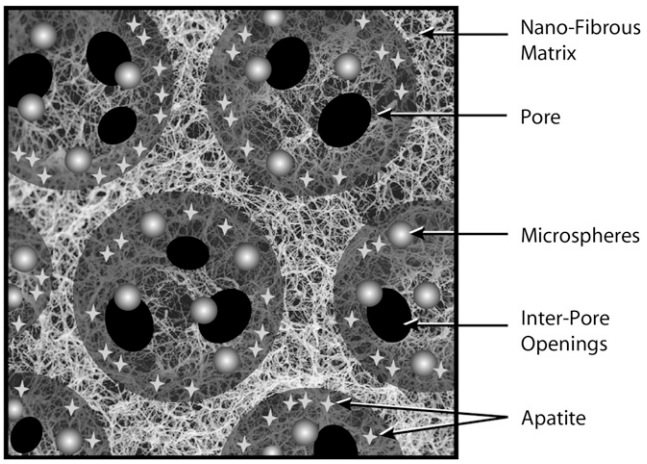

FIGURE 1 Biomimetic nano-scaffold for application in periodontal bioengineering. The scaffold combines novel nano-fibrous architecture of an interconnected pore network with microspheres for controlled release of putative regenerative factors. The nano-fibrous scaffolding design uses the architectural features of collagen, providing a high surface area for cell attachment and new matrix deposition, and an open structure allowing an interactive environment for cell–cell, cell–nutrient, and cell–signal molecule interactions. The bone mineral mimicking apatite enhances osteoconductivity of the scaffold. The biodegradable microspheres release the regenerative factors in a controlled manner in a targeted local environment. Adapted with permission from Elsevier (reference 26).

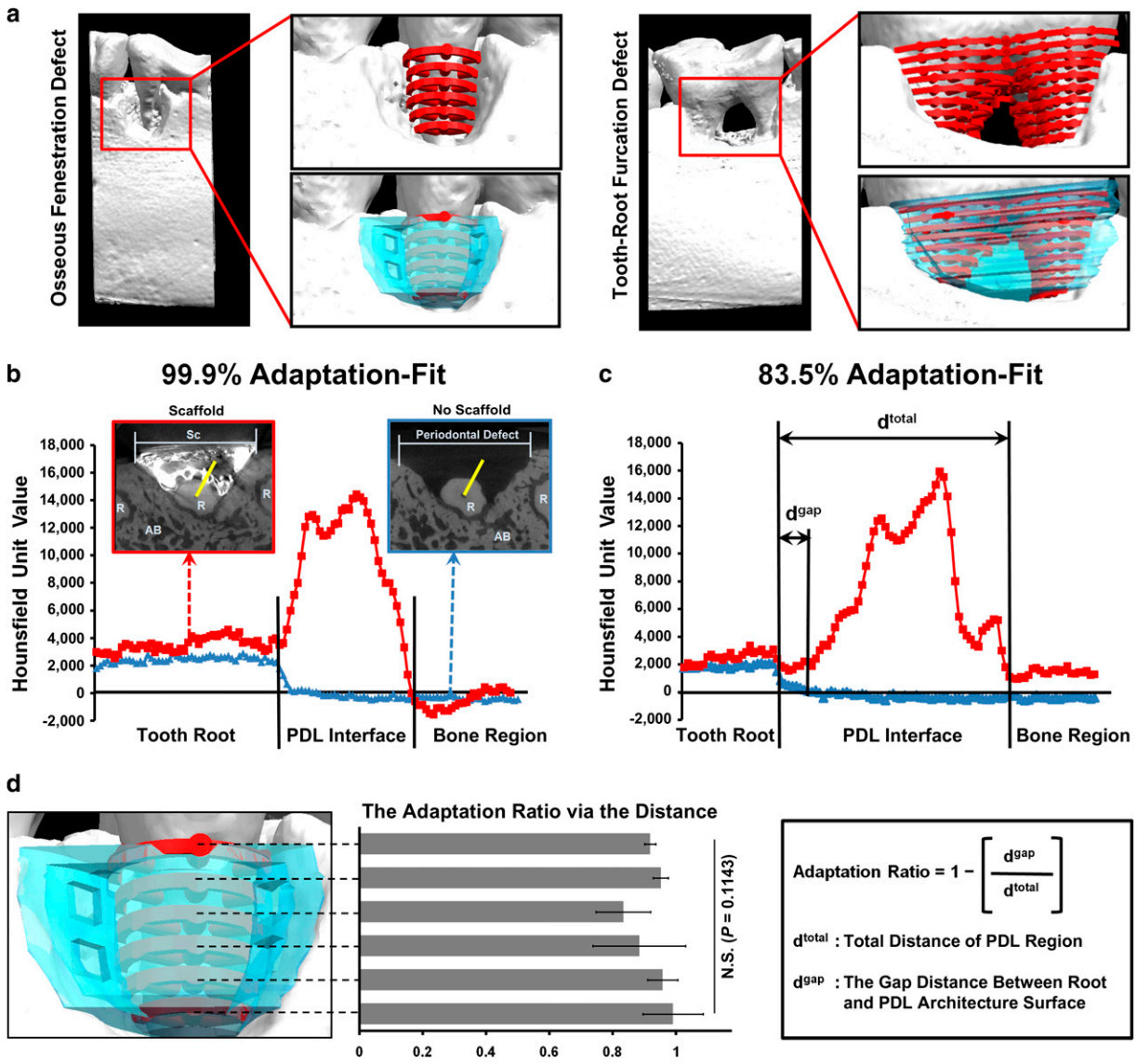

FIGURE 2 The reverse-engineered periodontal defect-fit scaffold modeling and the adaptation of customized designed scaffold on the root surface. **2a** Computer-aided design-based software (NX version 5 software, UGS, Plano, TX) was used to create PDL (red) and bone (blue) interfaces of the hybrid scaffold. The anatomic defect-fit scaffold had the perpendicular-oriented PDL internal channel structures and topological similarities of the periodontal defects. The furcation defect design had separated two different parts with a key (buccal)-lock (lingual) system to make easier assembling and implanting through the buccal–lingual penetration defect region. The red line was the porcine mandible image with the customized scaffold, and the blue line was the exposed periodontal defect site. **2b** The histogram represented the 99.9% adaptable scaffold to the root surface. The measured length was 3.00 mm, and the scaffold was coated by 35% $BaSO_4$ solution. The yellow lines on the two-dimensionally digitized slices represented the measured regions with 3.00 mm length from the dentin (dental pulp side) to the middle of defect site. AB = alveolar bone; R = tooth root; Sc = hybrid scaffold. **2c** The histogram was from 83.5% adaptable scaffold image. The concaved region of the red line can represent the gap distance (d^{gap}) between tooth root surface and PDL interface scaffold. **2d** Based on the method in 2c and 2d, total PDL interface length (d^{total}) and d^{gap} were linearly measured, and the adaptation ratio was calculated in each layer, which had three different channel-type structures. There was no statistically significant difference (N.S.) among six different layers, and the range of adaptation was 83.3% < mean value of adaptation ratio < 99.0%, and data were mean ± SD. For the statistical analysis, the non-parametric Kruskal-Wallis one-way analysis of variance test was used. Reproduced with permission from Elsevier (reference 33).

Emerging Technologies for Gene and Stem Cell Delivery Technologies to Enhance Bioavailability of Growth Factors

The use of gene therapy offers significant potential to target and deliver growth factor genes to periodontal defects.[35,36] Gene delivery has been shown to increase bioavailability, improve factor targeting to cells within osseous defects, and improve the overall delivery of growth factors to wounds in a more biologically relevant dose level compared to the "dose-dumping" formulations generally used in topical protein delivery of growth factors.[37] Growth factors such as PDGF have been used to repair periodontal[38,39] and peri-implant[40] bone defects, whereas BMPs have been used to treat a wider range of craniofacial defects, including the jaws, peri-implant defects,[41] extraction sockets, and periodontal lesions.[42] The use of gene delivery technologies has shown potential in the clinical arena to stimulate regeneration of chronic diabetic wounds in patients with neuropathic defects.[43,44] However, at this time, human

clinical data are not available for treatment of periodontal defects, only early-stage preclinical safety assessments.[38] Early studies for salivary gland repair have demonstrated proof-of-concept in the craniofacial complex.[45]

The use of cell delivery approaches has been reviewed recently for application to oral and periodontal wounds.[25] It has been long known that the PDL offers significant regenerative potential as a source of progenitor cells that can regenerate periodontal wounds.[46,47] The use of stem cell transplantation of PDL progenitors has been demonstrated in vivo in a variety of contexts.[48,49] Although promising in the administration of cells as local factories to drive production of newly formed tissues, the use of autologous cells for the repair of oral and periodontal wounds is quite limited, primarily from a practical standpoint.[50] However, the use of allogenic cell-delivery approaches has demonstrated significant potential in several human clinical trials to expand the zone of attached and keratinized gingiva through the production of local growth factors at the wound site.[51-54] A commercial product has been developed that uses cadaver-derived allogenic stem cells for application in local bone repair procedures.[55] Recently, cell transplantation of PDL progenitor cells has demonstrated the potential to form hybrid ligament–implant constructs (Fig. 3).[56,57] As such, there is significant potential for the use of either stem cells or PDL progenitor cells to form both soft and hard periodontal tissues in vivo. However, the practical challenges of regulatory, consistent cell populations from patient-to-patient and time required for procurement will be steps requiring significant optimization before being ready for the clinical arena in a real way.

Perspective

The significant advances in regenerative medicine offers some exciting opportunities for the reconstruction of complex periodontal defects. The field has grown significantly over the past decade, and the use of growth factors for application to periodontal and oral bone wounds is now a clinical reality. The future areas of development remain in delivery strategies to target growth factors to

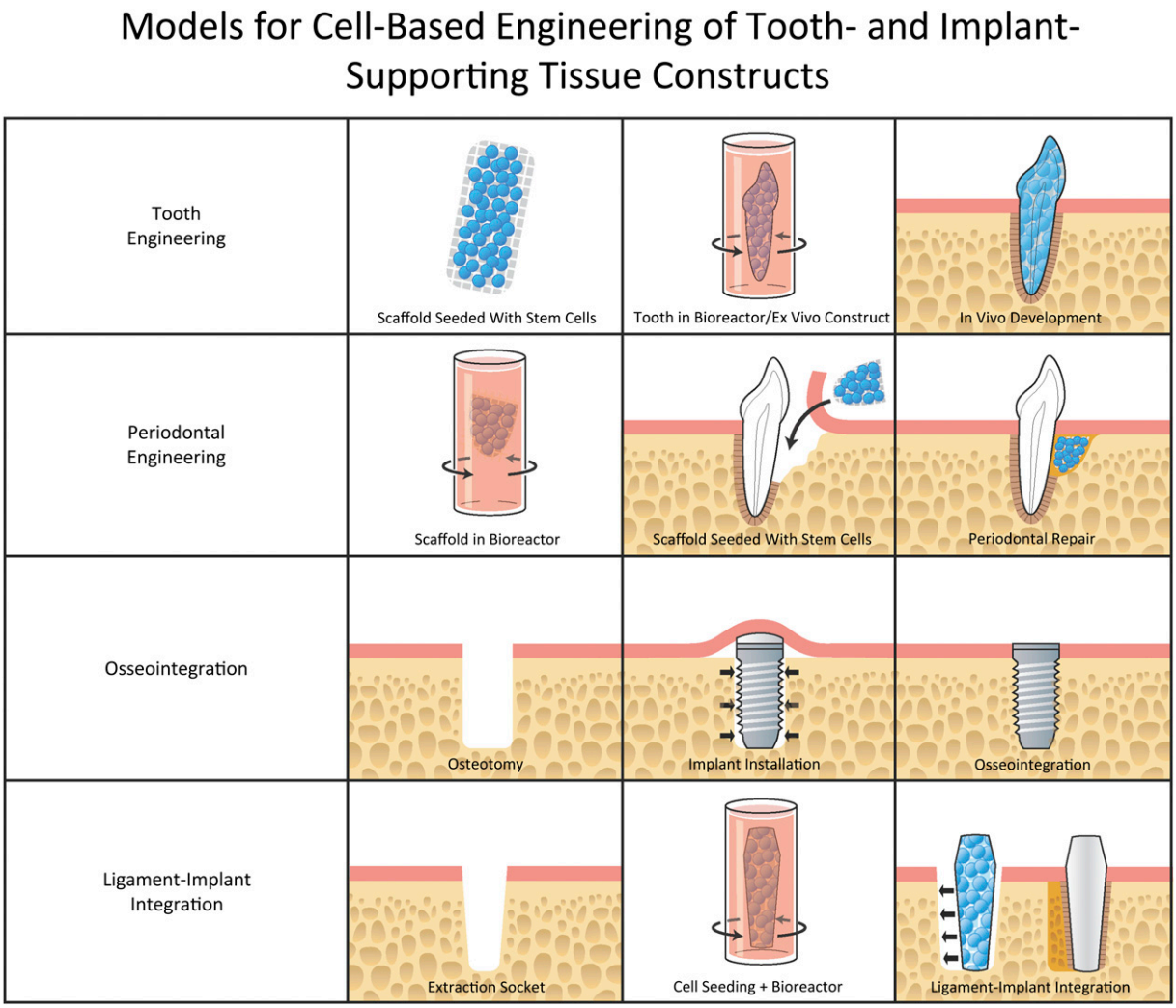

FIGURE 3 Cell-based therapies for the tissue engineering of teeth, periodontia, and dental implant interfaces. Reprinted with permission from Wiley-Blackwell (reference 57).

periodontal osseous defects. The use of optimized delivery vehicles will work on controlling the release and improving bioavailability (via improved scaffold designs, three-dimensional customized scaffolds, and possibly gene targeting). The use of stem or progenitor cells to improve cell sourcing to form new tissues and vasculature offers significant potential for robust tissue regeneration. These and other approaches will require important collaborations among biologists, engineers, and periodontal researches in concert with regulatory agencies and leaders to bring these new technologies to the chair-side and clinical practice. ■

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