BL Foster
MJ Somerman

Regenerating the periodontium: is there a magic formula?

Authors' affiliation:
B.L. Foster, M.J. Somerman, Department of Periodontics, School of Dentistry, University of Washington, Seattle, WA, USA

Correspondence to:
Dr Martha J. Somerman
Department of Periodontics,
School of Dentistry
Box 356365
University of Washington
Seattle
WA 98195
USA
Tel.: +1 206 543 5982
Fax: +1 206 616 2612
E-mail: somerman@u.washington.edu

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Structured Abstract

Authors – Foster BL, Somerman MJ

Objective – Left untreated, periodontal disease results in destruction of periodontal tissues including cementum, bone and the periodontal ligament, and subsequently, tooth loss. Increased research efforts focused on understanding periodontal disease at the cellular, molecular and clinical level have resulted in improved modalities for arresting disease progression; however, outcomes of existing procedures are not predictable and often disappointing. Critical to improving the predictability of regenerative therapies is targeting studies toward enhancing our understanding of the cellular and molecular events required to restore periodontal tissues.

Design – Toward this goal our laboratory has focused on defining cells, mechanisms and factors regulating development of periodontal tissues, using *in vitro* and *in vivo* rodent models.

Results and Conclusion – Results from these studies have enabled us to identify attractive candidate factors/cells including: 1) products secreted by epithelial cells that act on mesenchymal cells (amelogenins): we observed that both follicle cells and cementoblasts are responsive to amelogenin-like molecules resulting in changes in the expression of genes associated with cell maturation; 2) morphogens (bone morphogenetic proteins, BMP): we report that follicle cells respond differently to BMPs vs. cementoblasts, depending on dose of and specific BMP used; 3) phosphates: existing data suggest that phosphates act as signaling molecules regulating the expression of genes associated with cementoblast maturation. Knowledge gained from these studies has provided insight as to the cells/factors required for designing improved regenerative therapies.

Key words: amelogenins; bone morphogenetic proteins; cementoblasts; periodontal regeneration; phosphates

Introduction

Is there a magic formula? I imagine we all wish it was that simple and straightforward, but with the diversity in the activities of cells within the periodontium, coupled with the marked variation in the way individuals respond to insult, it is highly unlikely that one formula will fit all. Existing data from basic research and results from clinical studies in humans, whether case studies or clinical trials, provide convincing evidence that periodontal regeneration can be achieved, i.e. formation of new bone, new cementum and a supportive periodontal ligament (PDL). However, outcomes of existing regenerative therapies, while having positive results, are often disappointing when considering the extent of regeneration and furthermore, are not predictable. So, if there is a magic formula, our current knowledge base prevents us from defining the formula, although new knowledge gained from basic and clinical research has resulted in improvements in outcomes of regenerative therapies over the past decade.

As one approach toward defining the molecules critical for regenerating periodontal tissues our laboratory has designed experiments targeted at defining the cells/factors controlling periodontal tissue development with the belief that this tactic will provide insights into designing successful therapies for regenerating these tissues, as well as other mineral-associated tissues. As shown in Fig. 1 there are many features of the periodontium that need to be considered in attempts to restore lost tissues and many of these parallel development of these tissues, including: 1) cells involved in formation and in repair, e.g. cells involved in mineral homeostasis, cells evoked during an inflammatory process, cells promoting angiogenesis; 2) signaling molecules that control cell behavior; and 3) scaffolds controlling the release of identified factors.

Studies from our group as well as others have provided valuable information regarding factors to consider in attempts to improve existing regenerative therapies. Attractive molecules that have emerged include those reported to: promote osteoinduction, e.g. bone morphogenetic proteins (BMPs); enhance cell proliferation, e.g. platelet-derived growth factor; and display epithelial–mesenchymal (E–M) interactions during development, e.g. Emdogain®, enamel-like molecules, amelogenin, ameloblastin, parathyroid-related hormone protein (PTHrP) (1,2). Mechanisms controlling the effects of these molecules on periodontal tissues are complex and further, different factors even within the same family may exhibit diverse effects on the same tissue, with different effects *in vitro* vs. *in vivo*. Clues to defining the role of specific factors in regeneration of periodontal tissues have come from developmental studies directed at examining the expression of genes/proteins during development of the periodontium (3–5). This review describes factors that our laboratory considers to have some merit for use in regenerating periodontal tissues, including their known/suggested mechanism of action and the rationale for their consideration as candidate molecules for restoring the periodontium.

Approaches used to identify factors

Our laboratory has used a murine model of tooth development to identify timed and spatial expression of

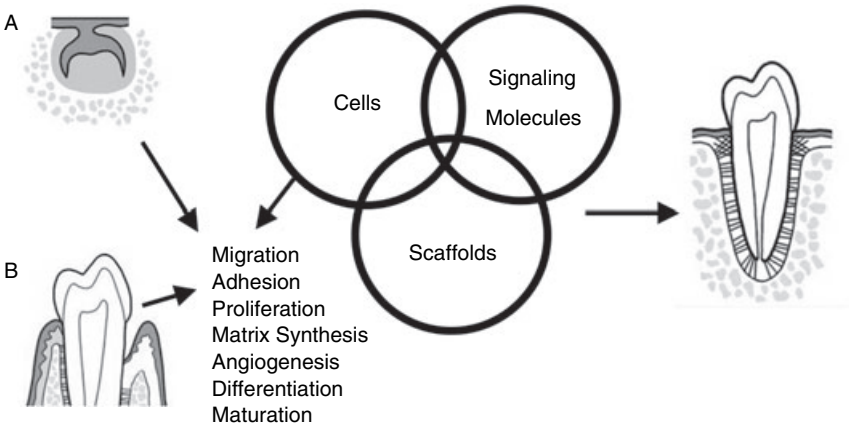

Fig. 1. Regeneration of periodontal tissues: issues to consider. Development (A) and regeneration (B) of periodontal tissues require similar cell activities and these behaviors may be initiated by the same signaling molecules. Once the specific factors required for regenerating tissues are confirmed then they need to be delivered to the local area over a designated time period. This requires designing appropriate scaffolds for incorporation and subsequent release of factors.

molecules considered to be regulators of cementogenesis. Results from these studies provided information that enabled us to begin to appreciate the molecules and cells controlling periodontal development and also, to develop *in vitro* models for further defining the role of follicle cells (the putative precursor cell of cementoblasts, osteoblasts and PDL cells) (6,7), PDL cells and cementoblasts. Procedures for isolation of these cells have been published (8,9). Briefly, we showed that during cementum formation, transcripts for bone sialoprotein (BSP) and osteocalcin (OCN), two mineralized tissue-specific molecules, are seen in cells lining the root surface and alveolar bone, cementoblasts and osteoblasts, respectively, but are not observed within the follicle region at early stages of root development or in the PDL region of the mature periodontium (8,9). The identification of specific factors expressed by cementoblasts, but not follicle cells or PDL cells, enabled us to isolate these populations and then to confirm the properties of the specific cell types *in vitro*. Next, a variety of experiments were designed to determine the responsiveness of these cells to molecules that had been identified by our group and others as compelling candidates for use in restoring periodontal tissues.

Defining the factors regulating formation of periodontal tissues

While it is evident that there are many factors that may prove important for controlling the PDL-mineralized tissue interface, this review focuses on three factors that our laboratory believes have a significant role in controlling the behavior of cells within the periodontium: 1) BMPs; 2) amelogenin-like molecules; and 3) phosphate-regulating factors (Table 1). The rationale for centering on these molecules and for their potential role as key modulators of periodontal tissue formation is provided below.

Bone morphogenetic proteins

Bone morphogenetic proteins are members of the transforming growth factor β superfamily. These secretory signal molecules have a variety of functions

Table 1. **Periodontal regeneration: candidate factors**

Factor	Suggested mechanism of action/function during development/regeneration of periodontal tissues	References (limited due to guidelines for 40 or less references)
1. Amelogenin-like molecules	Amelogenenins secreted by ameloblasts are considered to play a major role in regulating formation/crystal growth of enamel. More recent studies suggest that amelogenins, degradation/alternative spliced products, may act as epithelial–mesenchymal signaling molecules, i.e. regulate the behavior of odontoblasts and cementoblasts including cell maturation and mineralization.	(6,14–19,25–27,29,30)
2. Bone morphogenetic proteins	Certain BMPs are known to be involved in promoting cementoblast/osteoblast differentiation and subsequently mineralization. In contrast, evidence exists suggesting that other BMPs, e.g. BMP3 and/or the dose of BMP used may inhibit cell maturation and mineralization.	(3,7,10–13,37–40)
3. Phosphates	Existing data suggest that cells within the periodontal region are extremely sensitive to phosphate/pyrophosphate concentrations in the local environment and that phosphates may directly control expression of genes associated with the mature osteoblasts/cementoblasts.	(28,32,36)

during morphogenesis and cell differentiation (see review: 10). BMP2, 4, and 7 are co-distributed between ameloblasts (epithelial cells) and odontoblasts (mesenchymal cells) during tooth development, where they are considered to be part of the network of signaling molecules regulating initiation of crown formation (enamel–dentin) and cusp development (shape). In contrast, BMP3 expression appears to be limited to mesenchymal cells, with high expression detected in dental follicle cells during development and in PDL cells in the mature periodontium (3,11). Studies by Dr Lyons' group (12) indicate that BMP3 is an antagonist of osteogenic BMPs and mice lacking BMP3 exhibited twice as much trabecular bone as wild-type littermates, indicating that BMP3 is a negative regulator of bone density.

We have shown that follicle cells *in vitro* express genes for BMP2, 3, 4 and for BMP receptors BMPR-IA and BMPR-II and differentiation toward a cementoblast/osteoblast phenotype upon exposure to BMP2 (7). In contrast, exposure of mature cementoblasts to similar levels of BMP2 results in decreased expression of BSP, a major marker for the mature osteoblast/cementoblast and an inhibition of cementoblast-mediated mineral nodule formation (13).

Amelogenin-like molecules

Emdogain® (EMD; Biora/Straumann, Malmö, Sweden) is a product marketed for use in regenerating periodontal tissues based on the concept that E–M interactions are required for formation of cementum. EMD is an extract of low molecular weight porcine enamel proteins, containing predominantly amelogenin, and thus, the biological activity noted has been attributed to amelogenin (14). However, EMD contains other bioactive factors and may vary between preparations (14). Therefore, while effects of EMD on cell activity may be attributed largely to amelogenin they need to be interpreted cautiously as other factor(s) may be responsible for some of the activities observed. We and others have shown that EMD can alter the activity of follicle cells (15), cementoblasts (16), PDL cells (17) and osteoblasts (18), *in vitro*. In addition, while not predictable, impressive regenerative results have been reported when EMD was used to treat bone and periodontal defects in animal models and in patients (19). The use of an epithelial product to regenerate periodontal tissues is conceptually appealing. It is well established that E–M interactions are required for formation of the crown, i.e. enamel and dentin (20), and emerging data suggest that E–M interactions are required for development of periodontal tissues. Prospective candidates include amelogenin, ameloblastin (21) (also called amelin and sheathlin), PTHrP (22), laminin (23) and yet to be identified factors secreted by the epithelial root sheath (24). However, confirmation that E–M interactions are required for root formation is needed, as are data identifying the specific factors involved.

In collaboration with Drs Gibson and Kulkarni, using *in situ* hybridization, we have shown that mice null for amelogenin do not express BSP transcripts and have decreased expression for OCN (25). In support of this finding Shimizo et al. reported that the BSP promoter is activated by EMD (26). Also we noted a dramatic decrease in bone density and mandibular development when compared with wild-type controls, while Hatakeyama et al. have shown defective root development in null mice, including osteoclast-mediated root resorption (27). *In vitro*, exposure of follicle cells and cementoblasts to EMD or full-length amelogenin altered expression of both OCN (decreased) and OPN (increased) (15,16,28). These findings are consistent with results reported by Veis et al. (29), who identified two specific cDNAs comprised from amelogenin exons 2, 3, 4, 5, 6d, 7 and 2, 3, 5, 6d, 7 and revealed that the corresponding proteins, designated r[A + 4] (8.1 kDa) and r[A − 4] (6.9 kDa), enhanced transcripts for type II collagen, Sox 9 and Cbfa1 mRNA in embryonic rat muscle fibroblasts. *In vivo*, implantation assays demonstrated that these factors promoted expression of both BSP and BAG-75 protein. In more recent studies in collaboration with Drs Snead and Gibson we noted that in addition to amelogenin, cementoblasts *in vitro* treated with alternative spliced or degradative products of amelogenins (leucine-rich and tyrosine-rich amelogenin peptides) exhibit a similar response, i.e. a dramatic decrease in expression of OCN and an increase in expression of OPN (30). These data support a role for enamel matrix proteins beyond biomineralization.

Phosphates

Research with animal models, where genes are mutated or deliberately knocked out, has lead to considerable understanding of the genes required for tissue

formation. In this regard, we and others have noted considerable alterations in periodontal tissues in mice having mutations in or knockout of genes associated with phosphate regulation. We noted that cementum formation was dramatically greater in both *ank*/*ank* (ANK, protein of mouse progressive ankylosis gene; ANKH-human homolog) and PC-1 (nucleoside triphosphate pyrophosphohydrolase) mutant mice, where these genes/proteins regulate the level of pyrophosphate (PPi) in the local environment, when compared with wild-type litter mates. In contrast, the PDL and surrounding alveolar bone appeared normal (28). On the contrary, mice null for tissue non-specific alkaline phosphatase (TNAP), an enzyme that functions as a PPi-ase (31), exhibit minimal or no acellular cementum formation (32).

Mutated PC-1 and decreased ePPi levels have been identified in a patient affected with severe periarticular and vascular calcification (33) and the rare disorder, autosomal-dominant craniometaphyseal dysplasia has been linked to mutations in the human ank gene (ANKH) (very similar to mouse) on chromosome 5. Affected individuals have marked osteosclerosis of the craniofacial bones, often with neurological defects, metaphyses of long bones are flared, but extracranial skeleton and joints are otherwise not affected (34,35). The TNAP null mouse human counterpart, hypophosphatasia, is a heritable disease manifested by rickets and osteomalacia, with subnormal levels of serum TNAP activity. There are six subtypes: perinatal, infantile, childhood, adult, odontohyphosphatasia and pseudohypophosphatasia (31). The perinatal form is the most severe with no mineralization in the skeleton. Death *in utero* or early after birth is predictable. The adult type is marked by frequent bone fractures and periodontal disease (exfoliation of teeth). There is a dental-specific form, odontohyphosphatasia, marked by abnormalities limited to teeth. For an extensive review of proteins regulating phosphate metabolism, please see the publication by Terkeltaub (36).

Clinical utility and implications

Amelogenins and other factors with epithelial–mesenchymal interactions

It is reasonable to postulate that a relatively insoluble molecule such as amelogenin may affect cells within the local environment; however, it is more difficult to rationalize that this molecule would affect bioactivity of cells at sites at a distance from the tooth site. However, data are accumulating to suggest that amelogenins can regulate the behavior of mesenchymal cells and thus act as signaling molecules. Regardless of the role such molecules play during development of periodontal tissues it is clear that cells within the periodontium respond to these factors. In order to appreciate the potential role for such molecules, future studies need to focus on identifying specific cell-surface receptors and downstream pathways activated, and subsequently the significance of these pathways for promoting regeneration of periodontal tissues.

Bone morphogenetic proteins

Results from numerous studies, *in vitro* and *in vivo* have shown that certain BMPs promote osteoblast maturation and induce mineral formation. In fact, recombinant human BMP2/absorbable collagen sponge was approved by the FDA this year for treatment of tibia fractures and anterior spine fusion in the US. Nevertheless the results from periodontal regenerative/craniofacial investigations have been varied, with some positive results (37), but often unimpressive with reports of ankylosis (38–40). The results from our *in vitro* studies highlight the complexity of cells within the local environment and their responsiveness, which may vary for several reasons including the dose of and specific BMP used.

Phosphates

We believe that research targeted at establishing the properties of cementum formed in *ank*/*ank* mutant mice, coupled with studies defining the downstream effects of phosphates on genes controlling cell differentiation, will provide novel insights into mechanisms controlling mineralization. The information gained will be vital for determining the etiology of diseases associated with ectopic calcification and also, will improve the effectiveness of periodontal regenerative therapies (neocementogenesis).

The more research that is done the more we appreciate the multiplicity of responses of cells within the periodontium to specific factors. The technologies and animal models now available, such as microarray

analysis with detailed software for interpreting data, transgenic mice and real-time RT-PCR, allow us to gain information on the responsiveness of cells to factors in a timely fashion. The knowledge gained from these studies will assist in designing predicable regenerative therapies.

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