Exploiting the Bioactive Properties of the Dentin-Pulp Complex in Regenerative Endodontics

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Abstract

Introduction: The development of regenerative endodontic therapies offers exciting opportunities for future improvements in treatment outcomes. **Methods:** Advances in our understanding of regenerative events at the molecular and cellular levels are helping to underpin development of these therapies, although the various strategies differ in the translational challenges they pose. The identification of a variety of bioactive molecules, including growth factors, cytokines, chemokines, and matrix molecules, sequestered within dentin and dental pulp provides the opportunity to present key signaling molecules promoting reparative and regenerative events after injury. **Results and Conclusions:** The protection of the biological activity of these molecules by mineral in dentin before their release allows a continuing supply of these molecules, while avoiding the short half-life and the non-human origin of exogenous molecules. The ready release of these bioactive molecules by the various tissue preparation agents, medicaments, and materials commonly used in endodontics highlights the opportunities for translational regenerative strategies exploiting these molecules with little change to existing clinical practice. *(J Endod 2016;42:47–56)*

Key Words

Bioactive molecules, cell signaling, dentin, pulp, regenerative endodontics

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Endodontics is constantly evolving through improvements in our understanding of the basic sciences underpinning disease and its treatment, advances in materials and other technological innovations, together with the accumulation and sharing of clinical experience gained by practitioners. The combination of all of these various factors provides the greatest opportunities for improved clinical outcomes. Biologically based therapies have long been important in endodontics and offer significant promise for future developments. Historically, pulp capping and other approaches to pulpal wound healing can be traced back to at least the 18th century (1). More recently, however, advances in biology have allowed the basis of these approaches to be better understood and for novel, more targeted approaches to be pursued.

The term *regenerative endodontics* can be defined according to one's own perception as to what it encompasses. Perhaps in its least specific definition, it encompasses many of the different biologically based therapies aimed at stimulating pulpal wound healing. The American Association of Endodontists (AAE) defines regenerative endodontics as "biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp dentin complex" (2). This definition clearly states that the aim of these procedures is the replacement of lost cells and root structure without making the claim that this replacement is a complete recapitulation of the once lost tissue. However, a much narrower definition could be used that implies that these procedures must induce the regeneration of new tissue resembling the native pulp-dentin complex at the histologic level with the expected physiological functions; however, this seems an unlikely outcome of current regenerative endodontic approaches (3). Reports of endodontic procedures that use an intracanal antibiotic paste containing metronidazole and ciprofloxacin (double antibiotic paste) (4) or a triple antibiotic paste (5) combined with early investigations of the role of the blood clot in pulpal healing (6) have helped to develop clinical regenerative endodontics in recent years. A variety of chair-side procedures involving creation of a blood clot in a diseased pulp have now been reported and are often described as revascularization procedures (7). These reports have all contributed to a call for action to develop regenerative endodontic therapies for clinical use (2) and initiatives from the AAE to collate experience and support development of regenerative endodontics in clinical practice. There are now several examples of variations of the original clinical revascularization procedures that include the use of other scaffolds such as platelet-rich plasma (8), platelet-rich fibrin (9), and gel foam (10), as well as the application of exogenous growth factors such as fibroblast growth factor 2 (FGF-2) (10). These variations, among other developments, highlight the rapid evolution of the field of regenerative endodontics and the translational nature of its propelling research. Although there is no consensus definition of regenerative endodontics, the exciting future exploitation of this area may be best served by the adoption of a less strict definition for regenerative endodontics and the avoidance of semantic debates. In this way, clinical practice can keep progressing with the application of regenerative procedures that focus on meaningful patient-centered outcomes such as resolution of the disease and tooth survival. However, we recognize complete regeneration of a pulp-dentin complex that resembles the native lost tissue remains the ultimate goal, and that it is the driving force of considerable research efforts that are moving the field of regenerative endodontics into future more sophisticated therapy modalities.

Indeed, the biological focus of the exciting research in regenerative endodontics has greatly advanced our understanding of this area. In this review, we focus specifically

on the involvement of bioactive molecules found in dentin-pulp, their potential contributions to reparative/regenerative events, and their potential clinical exploitation. Although targeting bioactive molecules within dentin-pulp represents only one facet of regenerative endodontics, it may potentially allow rapid progress to be achieved within this area in the short-term with only subtle changes to existing clinical practices.

Bioactive Properties of Dentin and Pulp

Traditionally, dentin has been regarded as a relatively inert mineralized connective tissue that shows minimal remodeling after formation and in the absence of disease. In contrast, pulp is considered to resemble a classic soft connective tissue in terms of displaying turnover and remodeling, although its more gelatinous consistency contrasts with the fibrous nature of many other connective tissues. Although these perceptions of dentin and pulp have long been clinically prevalent, it is important to recognize that dentin has been known to show bioactive properties for more than 4 decades. Demineralized dentin matrix was demonstrated to induce pulpal repair (11) and apical closure (12) in primates, and this inherent pro-mineralizing effect was reportedly due to bone morphogenetic protein (BMP) activity (13–16). Subsequently, the soluble pool of non-collagenous proteins in dentin, which is released during demineralization of the tissue, was reported to induce both reparative (17) and reactionary dentinogenesis (18, 19). Furthermore, the actions of transforming growth factor-β (TGF-β) and other BMP family members on induction of odontoblast terminal differentiation in tooth development could be replicated by preparations of soluble dentin non-collagenous proteins (20). Thus, the perceived inertness of dentin reflects the immobilization and sequestration of these bioactive molecules within the matrix. However, their subsequent dissolution such as during caries provides a local release of their bioactivity.

Earlier studies focused on minimally purified dental tissue preparations, and where characterization of the preparations was undertaken, the analytical techniques were constrained in their ability to resolve individual bioactive molecules. However, advances in understanding of the nature of these various bioactive molecules and the techniques used for their characterization have since allowed a diverse group of molecules to be identified, many of which were recently reviewed (21). This diverse group of molecules encompasses growth factors, chemokines, cytokines, extracellular matrix molecules, and bioactive peptides, reflecting the complexity of the cellular signaling events capable of being induced. The subsequent discussion of these bioactive molecules and their potential involvement in dentin-pulp regeneration will focus on the signaling involved in the cascade of biological events associated with regeneration rather than simply cataloguing the molecules present. Although considerable research has investigated the biological actions of individual molecules in dentin-pulp regeneration, the microenvironment at sites of tissue injury will reflect the local dissolution of a multitude of bioactive molecules; thus, it is important to note that the summation and indeed synergistic actions of these molecules may differ significantly from those when present individually (22). Although a diverse range of bioactive molecules are also found within the dental pulp, their long-term bioavailability may be constrained by more rapid turnover of the pulpal extracellular matrix and the fact that this source may be unavailable in cases of pulpal necrosis. Thus, dentin can be considered a reservoir of growth factors and other bioactive molecules with important roles in repair and regeneration (Table 1).

The inertness of dentin reflects the immobilization and sequestration or "fossilization" of the bioactive molecules within the matrix. In health, these molecules will largely remain in their "fossilized" state, and it is only when injury and disease occur that matrix dissolution can be observed, leading to local release of these bioactive molecules. This is perhaps an oversimplification because the mechanism of immobilization/association of different bioactive molecules within the dentin matrix varies. In some cases, these molecules are associated with the dentinal mineral phase by a relatively nonspecific, perhaps ionic binding. However, for other molecules, the binding may be more specific in nature (eg, the specific interaction of TGF-β1, although not other

TABLE 1. Key Growth Factors and Morphogens Present in Dentin Known to Play Important Roles in Regeneration and Repair

Key growth factors in dentin matrix	Regenerative function
TGF-β1 (23, 24)	Involved in primary odontoblastic differentiation (25, 26) and in promoting tertiary dentinogenesis (20)
TGF-β2 (23)	Its expression is upregulated on differentiation of DPSCs into a mineralizing phenotype (27)
TGF-β3	Promotes odontoblastic differentiation (28, 29)
BMP-2 (30)	Promotes odontoblastic differentiation in both *in vitro* and *in vivo* models (31) and the induction of DSPP and increases alkaline phosphatase activity (32)
BMP-4 (30)	Increases odontoblastic differentiation (33)
BMP-7 (34)	Promotes mineralizing phenotype in DPSCs (35, 36)
Insulin growth factor-1 (37, 38)	Promotes proliferation and differentiation of DPSCs and SCAP into a mineralizing phenotype (39, 40)
Hepatocyte growth factor (41)	Promotes migration, proliferation, and survival of MSCs (42)
VEGF (24, 43)	Potent angiogenic factor (44–46) that has been shown to promote blood vessel formation in tooth slices implanted subcutaneously in SCID mice (47)
Adrenomedullin (48, 49)	Promotes odontoblastic differentiation through activation of p38 (22)
FGF-2 (24, 43)	Promotes stem cell homing (chemotaxis), stemness, and angiogenesis (44)
Platelet-derived growth factor (23)	Promotes angiogenesis (50), chemotaxis of MSCs (51), modulates the process of odontoblastic differentiation (52), acting synergistically with other growth factors (53)
Epidermal growth factor (43)	Enhances neurogenic differentiation of DPSCs (54) and SCAP (55)
Placenta growth factor (43)	Promotes angiogenesis (44) and osteogenic differentiation of MSCs (56)
Brian-derived neurotrophic factor (38)	Promotes neuronal growth and axonal targeting (57)
Glial cell line–derived neurotrophic factor (38)	Promotes nerve regeneration *in vivo* (58) and pulp cell survival/proliferation (59). Increased in expression during odontogenic differentiation (60).
Growth/differentiation factor 15 (38)	Promotes axonal regeneration and function after injury and plays important role in neuronal maintenance (61)