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)

isoforms, with decorin/biglycan in dentin) (62). Thus, dissolution of different bioactive molecules from dentin may occur over a range of conditions that permit modulation of the release of these molecules. Our understanding of the different tissue pools of various molecules in dentin-pulp and their relative solubility is still very limited; however, a better understanding could provide a powerful means to enable release of these molecules to stimulate natural repair processes.

Biological Events Associated with Regeneration

Repair and regeneration of dentin-pulp comprise a cascade of cellular events with matrigenic, angiogenic, and neurogenic outcomes, reflecting the various processes associated with generation, homeostasis, and function of these tissues. In natural pulpal wound healing, all of these processes are initiated and controlled by a variety of signaling molecules derived from the matrices of dentin-pulp as well as defense, inflammatory, and immune cells associated with disease and its progression. To understand the potential scope of dentin-pulp derived bioactive molecules in repair/regeneration, it is helpful to consider the cascade of cellular processes and events taking place (Fig. 1).

The physiological embryonic development of the dentin-pulp complex is coordinated by an exquisite blueprint of processes under tight temporospatial control (26). Conversely, the postnatal repair and regeneration of these tissues show much less degree of regulation. In part, the less controlled environment of repair and regeneration is a consequence of the exposure and release of a variety of bioactive molecules with potent cellular signaling properties from dentin-pulp in addition to the interplay with the inflammatory process (63). The more limited temporospatial and dosage regulation of signaling molecules, as compared with physiological tooth development, emphasizes the pathologic nature of events occurring during repair/regeneration. This perhaps helps to explain why clinical regeneration of a truly physiological-like pulp tissue is a daunting goal. The complexity of the cellular signaling environment is further exacerbated by the dynamics of the disease process on the release and exposure of bioactive molecules in dentin-pulp and their functional modifications through partial degradation by the acidic and enzymatic activities associated with disease progression (64, 65). Furthermore, surgical intervention by the clinician with endodontic irrigants, disinfectants, medicaments, and other agents will likely also modulate the signaling environment. We will describe how this complex and variable signaling environment may influence cellular events after injury and offers exciting opportunities for development of new therapeutic approaches that exploit the bioactive molecules in dentin-pulp. Although correlation of specific bioactive molecules with the different signaling steps associated with repair/regeneration is a laudable goal, some of these steps may involve networks of molecules, and also, more than 1 molecule may be able to duplicate the same action for some steps, reflecting either biological redundancy or other nuances of signaling.

Pulp Cell Niche and Stem Cell Behavior

Stem cells reside within niches, which provide a microenvironment responsible for maintaining the cells in their stem-like, undifferentiated state. This niche is determined by a complex interplay between the stem cells themselves, surrounding cells of various lineages, extracellular matrix, and soluble molecules including growth factors. These niches are generally regarded as providing relatively stable microenvironments, which likely only undergo significant disruption during disease and tissue remodeling. Although the concept of the niche is well-established, our understanding of its nature and behavioral influences on stem cells is still limited. These niches are often described by various markers known to be expressed at these sites, although the functional importance of many of these markers remains unclear. However, Notch signaling has been proposed to be important in controlling stem cell fate in the dental pulp after tooth injury (66). In the dental pulp, a perivascular stem cell niche has been reported (67, 68), which perhaps reflects the transient movement of vasculature-derived stem cells to the pulp. The prominent compartmentalization of stem cells on blood vessels within the apical papilla (69) and inflamed periapical tissues (70, 71) also indicates that perivascular stem cells participate in current regenerative procedures that have been shown to recruit cells from the apical region in both immature (72) and mature teeth (71).

Many of the bioactive molecules present in dentin-pulp have the potential to influence stem cell niches, although our understanding of these interactions is limited. Growth of pulp cells on a layer of isolated pulp extracellular matrix was shown to decrease their proliferation rate and favor expression of a stem cell–like phenotype (73). Furthermore, when these cells were grown in mineralization-inducing conditions, the pulp matrix allowed increased mineralization. Indeed, it has been demonstrated that the "stemness" of mesenchymal stem cells (MSCs) can be prolonged in culture when these cells are cultured on an extracellular matrix that mimics their native niche (74). Collectively, these studies demonstrate that the niche with its rich array of attachment and bioactive molecules has the ability to maintain stem cells at their

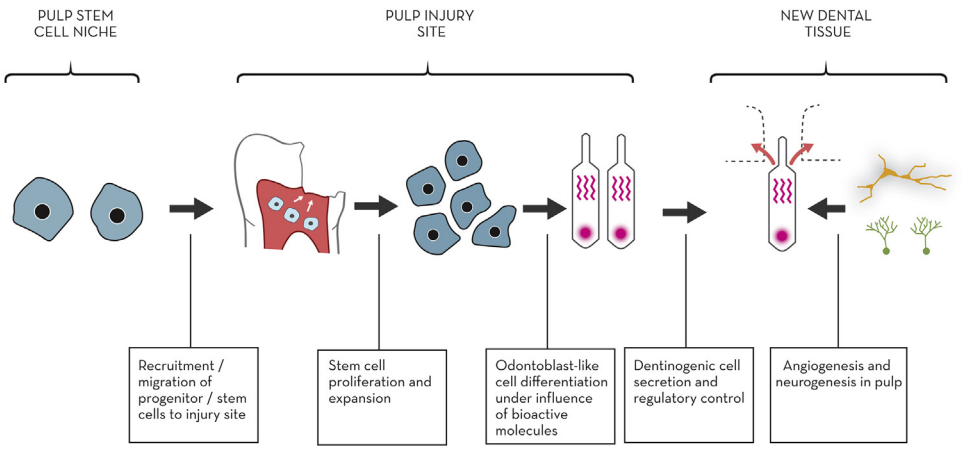

Figure 1. Schematic of the cascade of biological steps associated with healing events during dentin-pulp regeneration.

maximum differentiation potential. However, there is still much to learn of the cell-matrix interactions controlling pulp stem cell behavior.

Pulp Stem/Progenitor Cell Recruitment

Recruitment of stem/progenitor cells from their perivascular or other niches is a critical step in regeneration and guided tissue repair after injury. Release of chemotactic molecules, such as dentin or pulp matrix-derived molecules, at sites of tissue injury may be important to the recruitment process (Fig. 2). Perivascular niches for stem cells are attractive sources for recruitment after carious injury because of the close proximity of much of the pulp vasculature to the odontoblast layer (75, 76). Both dentin and pulp matrices have been reported to contain molecules with chemotactic properties (53, 65, 77), and although some of this activity may be ascribed to growth factors with known cell homing properties (53), other molecules are also likely to be involved. For example, complement activation and generation of C5a has been reported to be one of the molecules associated with lipopolysaccharide-induced pulp progenitor cell recruitment (77).

The target cell specificity of these chemotactic molecules in dentin and pulp is still not well-understood. Dentin matrix proteins can induce recruitment of cells involved in the inflammatory and immune responses of the pulp after injury (78) as well as pulp cells (65), and in the latter report, preferential attraction of cells expressing a range of stem cell markers was observed. Matrix-resident chemotactic molecules provide attractive candidate mediators for stem/progenitor cell recruitment because their release during carious tissue dissolution (18, 79) and irrigants like EDTA (24, 80, 81) used during endodontic treatment will likely lead to chemotactic gradients providing cues for the spatial localization of stem cells along dentinal walls in regenerative endodontic procedures (Fig. 2).

Pulp Stem/Progenitor Cell Expansion

Little consensus exists as to how many stem/progenitor cells exist within the dental pulp or even how many are required during repair and regeneration after tissue injury. However, it may be expected that the numbers of resident stem cells are relatively small, and some *in situ* or *ex vivo* expansion of their numbers may be required during repair and regeneration. Interestingly, the apical papilla (82) and the inflamed periapical tissues (70) appear to be enriched sources of undifferentiated stem cells that may play an important role in regenerative endodontics, particularly in immature teeth with open apices. Many of the bioactive molecules identified within dentin and pulp show proliferative effects on a variety of cell types including stem cells (73). Some of these activities may be ascribed to the various growth factors within dentin-pulp (15), although other molecules resident in these matrices will likely also contribute to such activities. Although it is valuable to try and catalogue all of the individual bioactive molecules demonstrating *in vitro* proliferative activities on various pulp cell populations in dentin-pulp, caution must be taken in extrapolation of such data to the *in vivo* situation. After injury to the pulp, there will be a complex cascade of cellular events taking place involving an intricate interplay of many bioactive molecules, including inflammatory mediators, with cell signaling properties (83). Anticipation of the outcomes of these interplays of molecules can be very difficult because it is not only the summation of the individual proliferative activities of these molecules that drives the outcomes but also the influence of various autocrine and paracrine regulatory factors in the *in vivo* tissue environment. Attempts to investigate proliferative events *in vivo* during odontoblast-like cell differentiation after tooth injury have clearly demonstrated the active nature of these events (84). However, there is still much to learn of the details of the signaling of these proliferative events and their regulation.

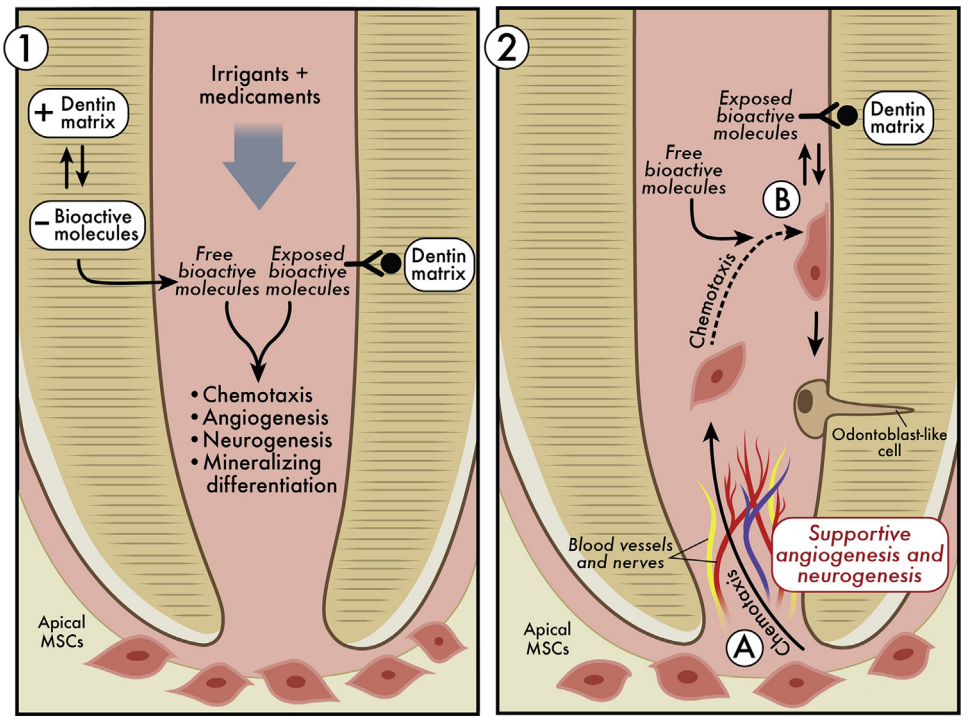

Figure 2. Schematic illustrating the potential actions of irrigants and medicaments in the release and/or exposure of bioactive molecules sequestered in dentin and their influences on regenerative events including chemotaxis, odontoblast-like cell differentiation, mineralization, angiogenesis, and neurogenesis.

Dentinogenic Cell Differentiation

Localized odontoblast cell death often follows moderate to severe dental trauma or carious injuries. Recruitment and differentiation of a new generation of odontoblast-like cells may subsequently ensue, allowing reparative dentinogenesis to occur, such as is seen in situations of mineralized bridge formation after pulp-capping procedures (17).

Differentiation of odontoblast-like cells during reparative dentinogenesis shows a number of parallels with the terminal differentiation of primary odontoblasts during tooth development (25). However, it must be recognized that differentiation of a new generation of odontoblast-like cells is a pathologic rather than physiological event, and the tightly regulated temporospatial control of odontoblast differentiation seen during tooth development is lacking. As a consequence, there may be considerable heterogeneity in the phenotypes of odontoblast-like cells and the matrices they secrete. At the morphologic level, this may be seen as variations in the tubular nature of dentin (85). Also, there appears to be differences in mineral composition of that formed by different MSC populations such as dental pulp stem cells (DPSCs) and stem cells of the apical papilla (SCAP), suggesting also heterogeneity that is based on the cell type involved in the differentiation process (86).

There is a dearth of knowledge relating to the molecular markers identifying "true" odontoblast-like cells from other forms of mineralizing cells such as osteoblasts. Nevertheless, it is important to remember that odontoblast-like cells do not represent a single, well-defined phenotype, and this has crucial implications for their robust identification. Although primary odontoblasts express a profile of molecular markers, including nestin, dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP-1), and matrix extracellular phosphoglycoprotein among other markers, such a profile is not unique to odontoblasts, and relatively simple molecular characterization is not necessarily a robust means of identifying the odontoblast phenotype. The addition of other identification criteria such as cellular morphology and matrix morphology, especially regularity of tubular structure, can increase confidence, although the application of these additional criteria is not necessarily easy. For example, the morphology of an odontoblast varies through its secretory life cycle (87, 88), and the tubular density of dentin will vary at different points of its thickness because of odontoblast crowding as dentinogenesis proceeds in a pulpal direction. Therefore, it is easy to see that the widespread identification of odontoblast-cell differentiation during dentin-pulp repair/regeneration in many different published studies in this field may not be quite as robust as previously thought. In the majority of these studies, there is little doubt that differentiation of a mineralized cell phenotype occurred, but whether these cells truly share the same phenotype as their physiological counterparts, the primary odontoblasts, remains unresolved. In functional terms, these various odontoblast-like cells and the matrices they secrete likely provide satisfactory outcomes for clinical procedures aimed at restoring the functional integrity of injured dental tissues. However, they cannot be considered to provide true regeneration of physiological-like morphologies for these tissues, and our limited understanding of the factors driving their differentiation means their generation can be somewhat serendipitous.

To try and identify the key morphogenic molecular signals for odontoblast-like cell differentiation, it is useful to look to those events occurring during physiological tooth development. During the late bell stage, inner enamel epithelium-derived growth factor signals, immobilized on the dental basement membrane, are presented to the peripheral cells of the dental papilla, leading to their terminal differentiation to odontoblasts (89). TGF-β superfamily members appear to be responsible for signaling odontoblast terminal differentiation physiologically and experimentally, because only immobilized TGF-β1 and TGF-β3 or a combination of FGF-1 and TGF-β1 could stimulate the differentiation of functional odontoblasts over extended areas of the dental papilla and allow for maintenance of gradients of differentiation (20, 90, 91). The ability of preparations of soluble dentin matrix proteins to mimic the effects of these growth factors on experimental embryonic odontoblast differentiation (20) also concurs with the effects of such dentin matrix preparations on odontoblast-like cell differentiation and reparative dentinogenesis in pulp-capping applications (17). The effects of dentin matrix components on the induction of dentinogenesis in pulp-capping situations have long been recognized (11, 17, 92) and may be due to the presence of a range of growth factors in dentin matrix preparations, including members of the TGF-β superfamily (14, 23, 37, 41, 43, 93) (Fig. 3). This is supported by reports of tissue-isolated and recombinant growth factors paralleling the actions of dentin matrix preparations (14, 15, 94). However, other components of dentin and pulp may also display morphogenic activity including extracellular matrix molecules such as DMP-1 (95) and BMPs (16, 20). Nonetheless, robust characterization approaches for the phenotype(s) of odontoblast-like cells and experimental functional deletion strategies are required to resolve which components

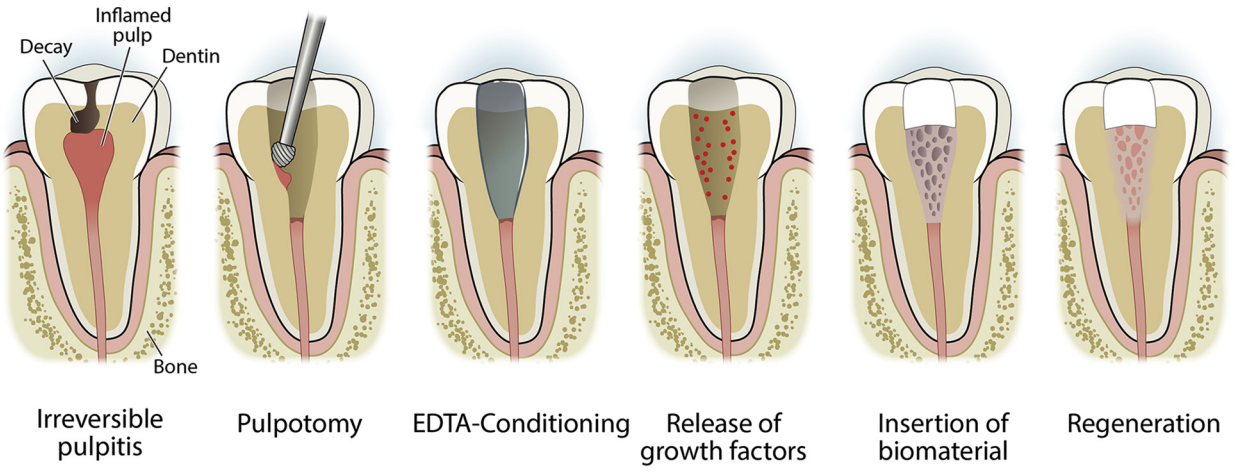

Figure 3. Illustration of the steps associated with clinical management of a tooth with irreversible pulpitis and the potential release of growth factors and other molecules leading to regeneration.

of dentin matrix preparations are essential for signaling odontoblast-like cell differentiation and to what extent the resultant cells resemble physiological primary odontoblasts.

Dentinogenic Cell Secretion and Its Control

Focus is generally centered on cell differentiation during repair/regeneration in dentin-pulp, but upregulation and control of subsequent secretion by the differentiated cell are also important. Control of dentin secretion occurs physiologically, with downregulation after completion of primary dentinogenesis and upregulation again occurring during episodes of tertiary dentinogenesis. In the absence of regulation of odontoblast secretion, pulp canal obliteration can ensue, with significant implications for both tooth vitality and endodontic treatment (96). Members of the TGF-β superfamily are capable of upregulating odontoblast matrix secretion under both physiological conditions (20) and in an *in vitro* repair/regeneration model (93). The target for this cell signaling appears to be activation of the mitogen-activated protein kinase pathway through p38 phosphorylation (22). Identification of this molecular switch for odontoblast secretory activity offers exciting opportunities to clinically modulate such activity through combinations of local targeting with growth factor stimulation or pharmacologic manipulation leading to a more controlled rate of mineral deposition.

Angiogenesis and Neurogenesis

Both the rich vasculature and a well-developed neural network in the pulp are important for its regeneration and function. After injury or chemical dentin conditioning, various proangiogenic growth factors sequestered in dentin (43) may be mobilized. Their proangiogenic activities (97, 98) may well contribute to local increases in vasculature to support reparative/regenerative events (Figs. 1 and 2). It is well-accepted that angiogenesis is crucial for regenerative procedures because despite being well-equipped to thrive in hypoxic environments (55), stem cells require adequate nutrient supply and gaseous exchange, particularly when in high metabolically demand secretory phases post-differentiation (99). Histologic evidence from both animal models (100–102) and clinical cases of revascularization procedures (103, 104) demonstrates that a good blood supply appears to be present after these procedures. This could be due to the angiogenic factors present on the dentinal matrix and released after the use of EDTA in revascularization procedures (24). In addition, MSCs when found in the hypoxic root canal system can release enhanced concentrations of angiogenic factors such as vascular endothelial growth factor (VEGF) (45, 105). Therefore, current procedures are known to promote robust angiogenesis and are likely driven by locally released growth factors from dentin and recruited stem cells.

The human dental pulp is richly innervated by primarily nociceptors, including neurons expressing potent neuropeptides such as calcitonin gene-related peptide, substance P, and neuropeptide Y (106). On pulpal injury, there is significant neuronal sprouting to the area of injury and repair (107). This enriched innervation is suggestive of a modulatory effect of neurons on dentin-pulp repair. Indeed, re-innervation coincided with pulpal healing and tertiary dentin formation in a model of pulpal repair after replantation of teeth (108), and denervation via nerve transection or pharmacologic ablation results in aberrant dentin formation (109, 110).

Although our understanding of neurogenic events after injury and regenerative endodontic procedures is more limited, the variety of neuropeptides and neurotrophic molecules expressed by odontoblasts and surrounding fibroblasts appear to play a role in the recruitment of nearby free nerve terminals to the area of tissue repair/regeneration (106, 111–114). In addition to resident pulpal cells, recruited MSCs such as SCAP have been shown to mediate axonal sprouting and targeting through a brain-derived growth factor mechanism (57). Interestingly, in a dog model of regenerative endodontics, a subpopulation of DPSCs with increased expression of brain-derived neurotrophic factor was transplanted into empty root canals, resulting in the formation of an innervated pulp-like tissue (115, 116). Therefore, adequate innervation should be a goal of regenerative endodontics because neurons mediate protective nociception and the modulation of homeostasis processes such as inflammatory responses and odontoblastic function.

Infection, Inflammation, and Regeneration

As the local environment of the tooth changes and becomes more anaerobic, the carious bacterial biofilm composition becomes more complex as it drives through the enamel, dentin, and pulp (117). The dentin-pulp complex aims to defend itself from the bacterial onslaught, and in the early stages of dental tissue infection, the odontoblasts detect and respond to the microbial presence (118, 119). As the infection progresses, cells more centrally located within the pulp, including resident immune cells, pulpal fibroblasts, vascular endothelial cells, and even stem cells, are also able to mount a response aimed at containing the infection (120–122). A variety of microbial sensors have been described as being present on these cell types, and the best characterized are the Toll-like receptor family that can detect microbial components ranging from their nucleic acids to cell wall constituents (122). After detection of the infection by host cells, cytokines such as interleukin (IL)-1α, IL-1β, tumor necrosis factor alpha, IL-4, IL-6, IL-8, and IL-10 are secreted, and depending on levels and temporality, they can lead to several cellular and tissue outcomes (123, 124). At the early stages and/or at relatively low levels of infection, the inflammatory response may be sufficient to not only contain and remove the infection via the local release of antimicrobial peptides and reactive oxygen species (ROS) but may also stimulate dental tissue repair responses. Indeed, there is now significant evidence to indicate that cytokines and other proinflammatory mediators such as ROS can directly stimulate dental tissue repair responses. However, if the infection increases, there will be an elevation of the inflammatory response mediated by the cytokine signaling network generated from the local cells and from the demineralized dentin that also releases signaling molecules into the milieu (83). Combined, this will lead to increased recruitment of a range of inflammatory cells, including neutrophils, macrophages, T cells, B cells, and plasma cells, which aim to use their intracellular and extracellular armamentarium to contain and kill the invading bacteria (125–128). This localized protective response to tissue infection and injury aims to prepare the tissue for eventual repair, and ideally the inflammatory reaction should be short-lived. Indeed, if it is excessive or prolonged, it will likely result in tissue damage because of either the direct toxic effect of the bacteria or the exuberant host response that results in collateral tissue damage in its attempts to combat the infection.

Central to both the inflammatory and healing responses is the role of the vasculature and the induction of angiogenesis. As has previously been described, angiogenic events can be triggered by the release of potent signaling molecules from the demineralized dentin. Interestingly, many cytokines classically regarded as being fundamental to the inflammatory response, such as IL-1, IL-6, and IL-8, are also proangiogenic (129–131). This pleiotropism is potentially not surprising because of the need for a robust vascular response to enable the delivery of the immune cells to the dental tissue and the removal of unwanted material such as toxic molecules and metabolites. Indeed, the first 3

of the 4 cardinal signs of inflammation—redness, warmth, swelling, and pain—are attributable to the vascular response. To empower the immune and repair responses within the vasculature, adhesion of immune cells and platelets is activated, as is coagulation, thrombosis, and vascular permeability. These processes enable delivery of inflammatory cells to the diseased tissue while isolating the area and setting the stage for tissue repair and regeneration (132). Furthermore, a variety of MSC niches are reported locally within the dental pulp (eg, perivascularly), and clearly MSC mobilization and homing are highly dependent on the vascular and angiogenic responses. Further highlighting the importance of the angiogenic response in dental tissue defense and repair are the data demonstrating the ability of dental MSCs to robustly differentiate down endothelial cell lineages (45, 46, 133). Once recruited, dental MSCs not only have repair characteristics, but they are also immunomodulatory. Their properties include their ability to suppress proinflammatory cytokine levels while upregulating anti-inflammatory molecules, along with their ability to directly temper immune cell behavior (134, 135). It is also of significance that several chemokines and homing molecules are shared by immune cells and MSCs, indicating the importance of the crosstalk between these 2 arms of the tissue defense and repair responses (77, 136) (Fig. 4).

Data from a variety of fields, in particular in mineralized tissue wound healing, are now highlighting the interdependency between inflammation, angiogenesis, and regeneration. It appears that although low-grade or the early inflammatory response can promote tissue repair, as might be seen beneath calcium hydroxide–based or mineral trioxide restorations within the pulp (137–139), relatively high-grade chronic inflammation negatively impacts directly on MSC function and dentinogenic repair and can also delay angiogenesis (83, 140).

Translationally, the progressive influence of pulpal inflammation on healing creates significant diagnostic responsibility when planning regenerative procedures to select cases that are likely to respond to treatment. At present, pulpal inflammation is simply classified as reversible, symptomatic, or asymptomatic irreversible pulpitis (141), with the diagnosis established after pain history, clinical/radiographic examination, and pulpal sensibility tests. Traditionally it was believed that the results did not accurately reflect the true histopathologic status of the pulp (142–144) and that new molecular diagnostic test would be required for accurate diagnosis (145). Recently this has been questioned, and a strong correlation has been shown between symptomatology, clinical findings, and the histologic classification of pulp disease (146). However, to improve clinical outcome a better understanding of the interplay between the reparative processes at the molecular level and clinical signs and symptoms may enable the development of new therapeutic modalities for regenerative endodontics.

Exploiting Bioactive Molecules in Dentin-Pulp

Therapeutic application of a variety of bioactive molecules, whether of natural or synthetic origin, to the injured pulp to promote repair and regeneration has been proposed (147). Although such a strategy is attractive in that it parallels the well-established techniques used in pulp capping, a number of delivery and technical considerations must be addressed. These particularly focus on the preservation of biological activity in a group of labile molecules, which presents significant challenges for routine clinical use where their storage and delivery may be challenging. An alternative strategy is to exploit the bioactive molecules naturally sequestered within dentin. Those within dentin are protected while the tissue is in its mineralized state, and their local release at sites of injury avoids many of the problems of effective clinical delivery in the surgery. Some release of these molecules may occur as a consequence of carious demineralization of the tissue and has the potential to participate in natural regenerative events. However, more targeted

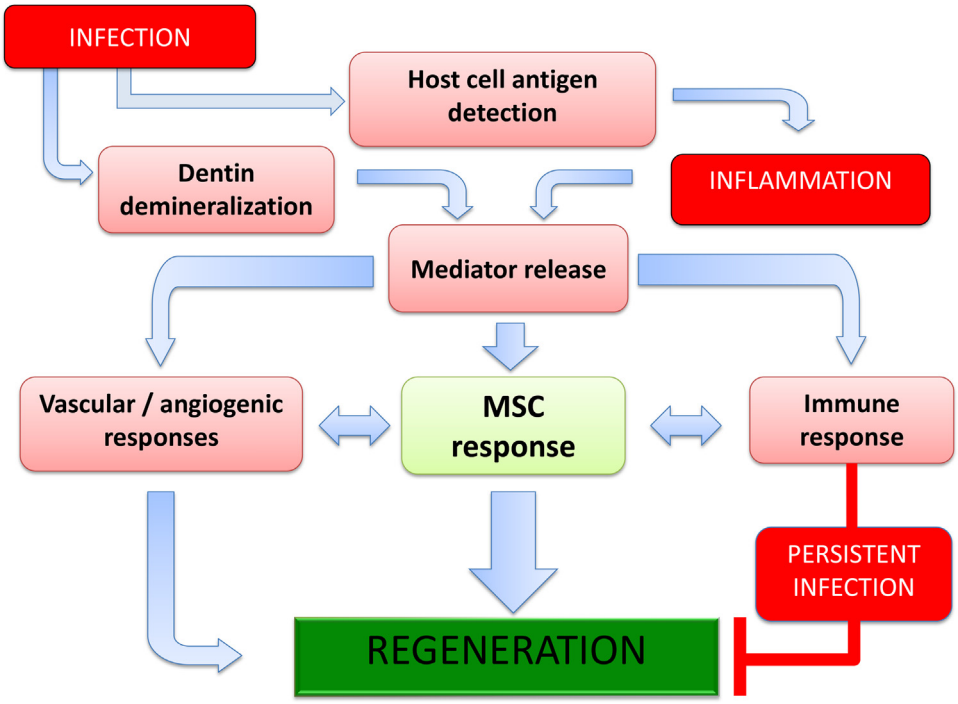

Figure 4. Schematic of potential steps and interactions between dental tissue infection, inflammation, vascular responses, and regeneration. An inflammatory response occurs generally after carious bacterial infection, leading to mediator release from host cells and demineralized dentin. This cocktail of cytokines, growth factors, and other signaling molecules acts on MSCs, immune cells, and the vascular system. There is significant crosstalk between these systems, and ultimately if the immune cell response is able to contain or remove the infection, potentially with the involvement of clinical intervention, then dental tissue regeneration can occur.

release might be achieved through local application of demineralizing agents, a number of which are commonly used for tissue preparation during endodontic procedures. Several studies have now highlighted the potential of various agents, epigenetic modifiers, medicaments, and materials to release and expose bioactive molecules in dentin to influence stem cell behavior (24, 49, 148–153). It is noteworthy that initial revascularization-like procedures were designed with the goal of maximum disinfection without taking into account the effect of the chemical debridement on the availability of bioactive molecules present in dentin and the survival and differentiation of stem cells. There is increasing evidence that disinfection can be achieved while promoting a microenvironment that is more suitable for regeneration. Thus, the strategy of targeting endogenous bioactive molecules sequestered in dentin can be achieved with minimal change to current endodontic practice while retaining the fundamental need for adequate disinfection, a pre-requirement for regeneration/repair.

Concluding Remarks

The future for regenerative endodontics offers exciting promise. In the long-term it is envisaged that regrowth of entire tooth structures within patients may be achievable; however, more short-term goals along that clinical and research journey are now becoming more realistic. Indeed, our increasing understanding of the molecules involved and cell behavior necessary for dentin-pulp complex repair will provide new therapeutic avenues for exploitation. These approaches may well be underpinned by the generation of biomimetic environments that harness the tooth's own natural ability to repair itself built on the basis of our understating of its innate biological properties. Subsequently, it is essential for continued and genuine clinical and basic science research partnerships to enable challenging questions to be asked and answered as well as to enable the translation of key research findings. These studies will be inherently interdisciplinary at the interface between both biological and physical sciences and in particular relating to biomaterials, cell scaffolds, molecular biology, and stem cell biology.

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