Vitality of the Dentin-Pulp Complex in Health and Disease: Growth Factors as Key Mediators

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Abstract: The vitality of the dentin-pulp complex, both during tissue homeostasis and after injury, is dependent on pulp cell activity and the signalling processes, which regulate the behavior of these cells. Research, particularly over the last ten to fifteen years, has led to a better understanding of the molecular control of cellular behavior. Growth factors play a pivotal role in signalling the events of tissue formation and repair in the dentin-pulp complex. Sequestration of growth factors in the dentin matrix during tissue formation provides a pool of these molecules, which may be released during injury and contribute to signalling of reparative events. Therapeutic intervention with recombinant growth factors also provides possibilities for control of cell activity during repair. Harnessing both endogenous and exogenous sources of growth factors can provide exciting opportunities for novel biological approaches to dental tissue repair and the blueprint for tissue engineering of the tooth. These approaches offer significant potential for improved clinical management of dental disease and maintenance of tooth vitality.

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Key words: growth factors, dentin, pulp, cell behavior, regeneration, tissue engineering

Submitted for publication 3/3/03; accepted 4/9/03

The vitality of the dentin-pulp complex is fundamental to the functional life of the tooth and is a priority for targeting clinical management strategies. Not only do the cells of the pulp maintain tissue homeostasis after tooth development, but they also underpin the defense reactions taking place in response to injury, such as caries, and the reparative events leading to tissue regeneration. Signalling of cells to control their behavior and activity is of critical importance to maintaining pulp vitality.

Responses of the Dentin-Pulp Complex to Injury

The overall response of the tooth to injury, such as dental caries, represents the complex interplay between injury, defense, and regenerative processes (Figure 1). Whilst each of these is often considered in isolation, it is important to recognize that the interplay and relative balance among these processes will be the primary determinant of tissue vitality and tooth survival.

Dental injury perhaps represents a special situation in that the cellular pulp is enclosed by a rigid, mineralized, tissue shell, and thus significant enamel and dentin matrix degradation can ensue before the disease process reaches the pulp. Nevertheless, the tubular structure of dentin confers significant permeability properties on the tissue,[1,2] and both bacterial metabolic products from the carious process and dental tissue matrix degradation products may diffuse down the dentinal tubules and invoke cellular responses.[3-8] With milder injury, cell necrosis may be limited; however, a number of molecular events will be initiated as a part of the pulp response to the injury. As the intensity of injury increases, these responses will magnify but will start to be offset by the effects on cell necrosis. Infection of the pulp itself will exacerbate the processes and provides particular challenges to the clinical management of the diseased pulp. The balance of all of these events will impact significantly on the opportunity and nature of any regenerative processes within the dentin-pulp complex.

Defense encompasses a variety of local tissue and systemic responses to counteract the injury.[9] These will include local inflammation within the tissues as well as local activation of immune defense reactions, which may trigger broader systemic immune responses. The role of bacteria in pulpal inflammation[10] and the tissue features of the inflammatory process[11,12] are well recognized. The scope of

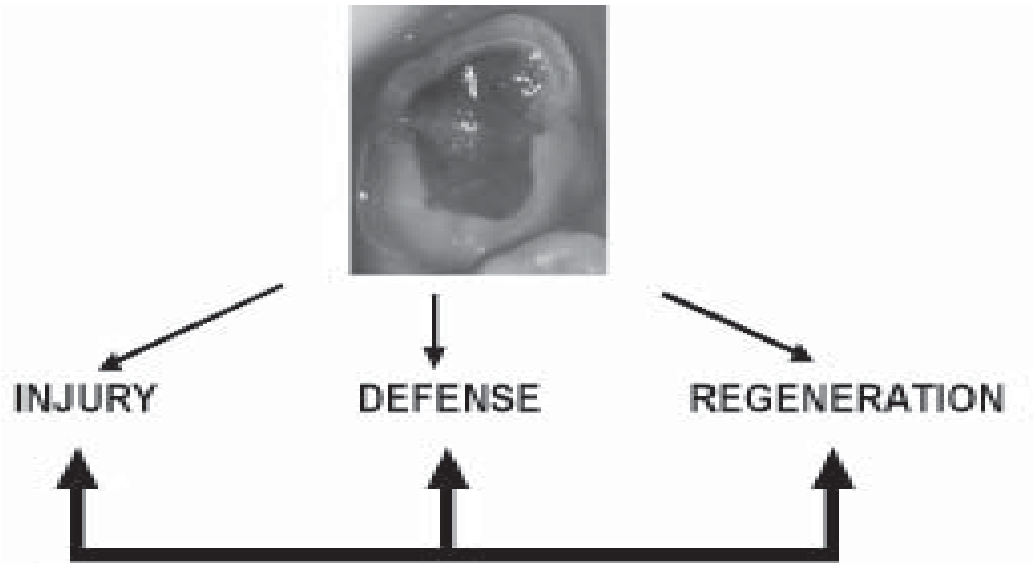


Figure 1. The overall response of the tooth to injury, such as dental caries, represents the complex interplay among injury, defense, and regeneration.

the immune defense reactions taking place is still not well understood, although a number of studies have highlighted the importance of these reactions. T lymphocytes and macrophages have been proposed to be important in the immunosurveillance of the pulp[13] and may be important in the initiation of pulpal specific immunity following exposure to protein antigens. B and T lymphocytes are found in carious pulps exhibiting reversible or irreversible pulpitis,[14] and increases in numbers of these cells have been correlated with increasing lesion depth in caries.[15,16] T lymphocytes are envisaged as signalling activation of other antigen-specific effector cells, such as B lymphocytes.[13]

While a histological view of inflammation in the carious pulp has long been recognized, our understanding of the molecular mechanisms of these events and their control is still in its infancy. Development of robust experimental models will be required to help us to elucidate these molecular pathways for signalling inflammation. Nevertheless, involvement of many of the well-recognized inflammatory pathways and molecular mediators is probable, and there is a need to characterize their roles within the context of the pulp and its environment.

Despite the apparent quiescence of odontoblast activity after primary dentinogenesis and tooth formation are complete, regeneration or wound healing in the form of tertiary dentinogenesis can ensue following injury and, of course, provides the basis for dentin bridge formation at sites of pulp exposure. Dentin bridge formation arises after odontoblast death at such sites and requires the differentiation of a new generation of odontoblast-like cells from pulp stem or progenitor cells. This involves a sequence of cellular events including stem cell recruitment, cytodifferentiation, and subsequent activation or up-regulation of the secretory activity of the cells (Figure 2) and has been termed reparative dentinogenesis.[17,18] In carious pulp exposures, bacterial irritation and inflammatory responses of the pulp may adversely affect reparative dentinogenesis and dentin bridge formation.[19,20] While such irritation and inflammation will be dependent on the disease activity of the lesion, it may compromise pulp vitality and opportunities for repair and regeneration to take place. When injury is of milder intensity (for example, early caries), odontoblasts are capable of surviving the injurious challenge and are up-regulated to secrete a tertiary dentin matrix at the pulp-dentin interface called reactionary dentin.[17,18] This acts to increase the barrier between the cells of the pulp and the injury. The cellular events during reactionary dentinogenesis are much less complex than during reparative dentinogenesis since dentin-secreting cells are already differentiated and their secretory activity simply has to be up-regulated.

Growth factors are a key group of molecules responsible for signalling a variety of cellular processes following dental injury. They play a central

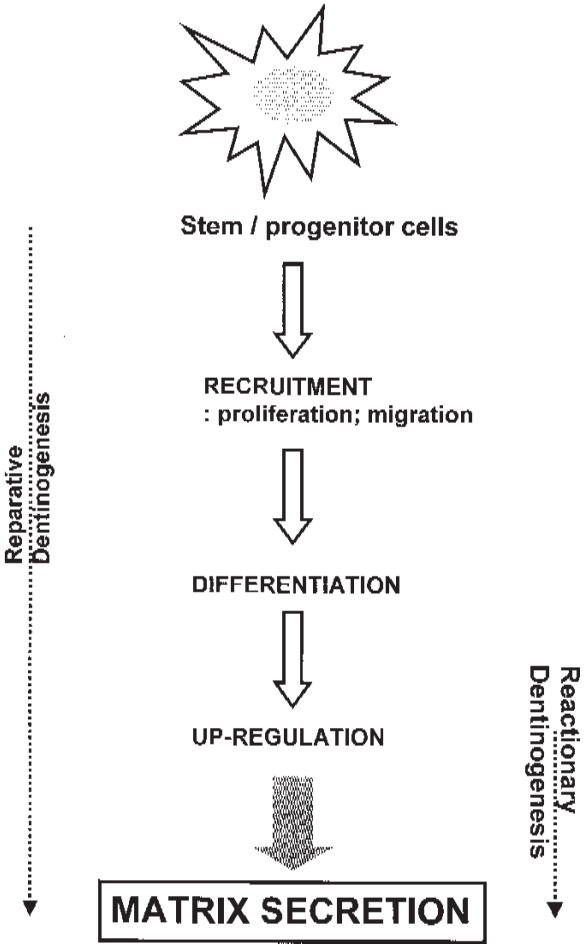


Figure 2. The cellular events involved in reparative and reactionary dentinogenesis

role in signalling various aspects of tissue regeneration, and the events associated with the initial injury to the tissue and subsequent defense reactions also impact on their activities. Importantly, they provide a basis to understand the biological mechanisms of tissue regeneration in the dentin-pulp complex and the effects of endodontic treatment, thereby offering exciting opportunities for novel treatment modalities and tissue engineering approaches to tissue restoration.

Growth Factors as Cell-Signalling Molecules

Growth factors are peptide molecules that transmit signals between cells functioning as stimulators and/or inhibitors of growth as well as modulators of differentiation state amongst other roles. As such, they play a central role in controlling cell behavior and activity. They may demonstrate a degree of specificity in terms of the cells they act upon, although some are more versatile and act on numerous cell types. The dose dependency of their effects also varies; however, one of the characteristic features of these molecules is their potency at very low concentrations, typically in the picogram range. The list of growth factors in Table 1, while not comprehensive, identifies some of the more commonly known factors. While many are named after the original source they were isolated from, this may be misleading because a more widespread distribution has often been since demonstrated.

Table 1. Superfamilies and families of the more commonly recognized growth factors

Superfamily	Family	Abbreviated name
Transforming growth factor β	Transforming growth factor βs	TGF-β
	Inhibins	Inhibin/Activin
	Bone morphogenetic proteins	BMP
	Vg-1	Vg-1, GDF-1, DPP
Platelet-derived growth factor	Platelet-derived growth factors	PDGF
	Vascular endothelial growth factors	VEGF
	Connective tissue growth factors	CTGF
Epidermal growth factor	Epidermal growth factor	EGF
	Transforming growth factor α	TGF α
Other large peptide growth factor families	Fibroblast growth factors	FGF
	Insulin-like growth factors	IGF
	Nerve growth factor	NGF
	Tumor necrosis factors*	TNF

*TNF-α and TNF-β are usually classified as pro-inflammatory cytokines, but sometimes considered within growth factor classifications.

Growth factors may act in endocrine, autocrine, paracrine, juxtacrine, and intracrine modes (Figure 3), highlighting the complexity of control of cellular activities in the body. They act through their interaction with specific receptors on the cell surface (Figure 4). Binding to these receptors leads to a chain of intracellular signals, the result of which is transduction of the signal to the cell nucleus. It is through their effects on gene expression in the cell nucleus, mediated by transcription and other factors, that the growth factors influence cell behavior and activity. This transcriptional control of gene expression can have far-reaching effects both in terms of intra- and extra-cellular events. Thus, growth factors may regulate genes controlling cell proliferation, cell differentiation, or the secretory products of the cell.

Growth Factors in the Dentin-Pulp Complex

While a variety of cytokines (cell-signalling molecules associated with inflammatory and immune reactions) have long been recognized to be associated with pulp responses to injury after dental caries, the roles of growth factors in cell signalling during tooth development and regeneration have only been recently identified.[9,21,22] Growth factors are responsible for signalling many of the key events in tooth morphogenesis and differentiation, and recapitulation of these processes after dental injury allows tissue regeneration.

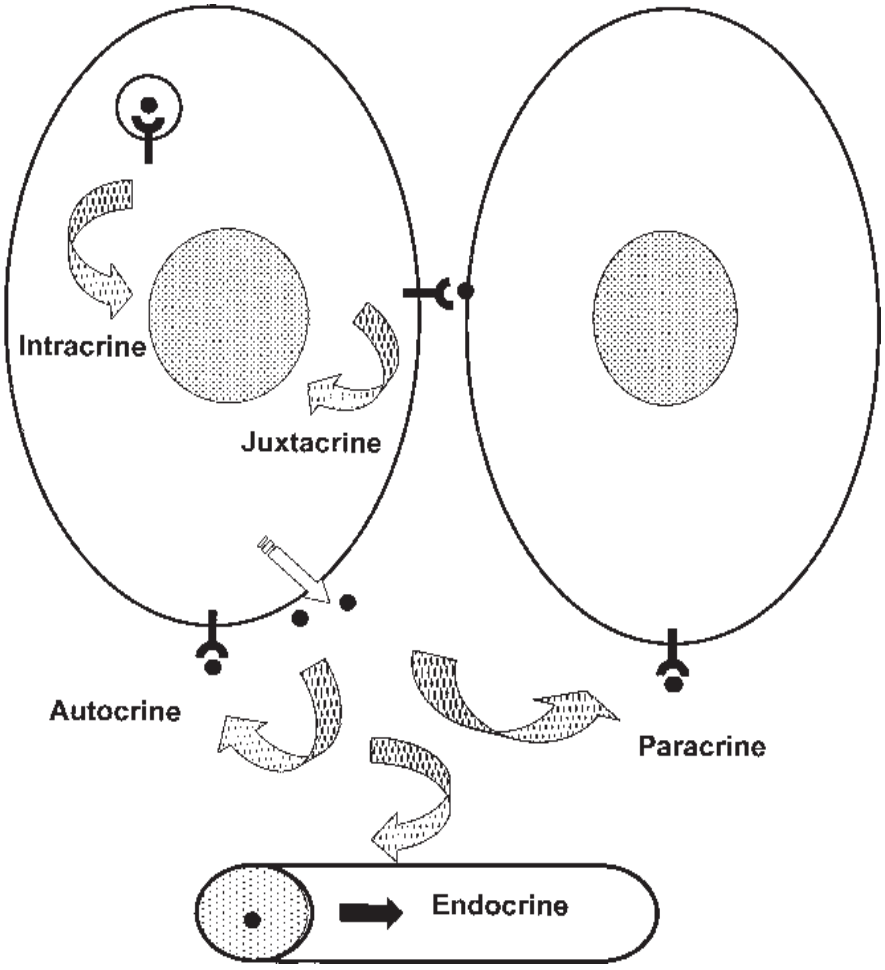


Figure 3. Growth factors may act in endocrine, autocrine, paracrine, juxtacrine, and intracrine modes. Growth factors (black circles) are produced and released from cells and then interact with (via specific receptors) either the cells producing them (autocrine, intracrine) or other cells (paracrine, juxtacrine, endocrine) resulting in the signalling of a response by these cells.

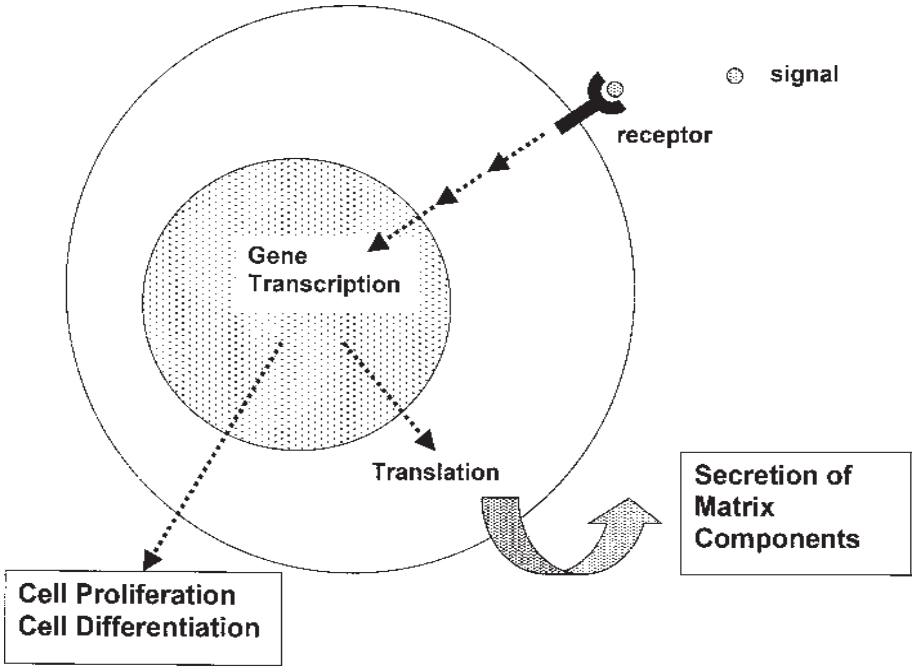


Figure 4. Growth factor interaction with a specific receptor on the cell surface results in signal transduction to the nucleus influencing gene transcription, which can direct a variety of responses including proliferation, differentiation, and matrix secretion.

During tooth development, epithelial-mesenchymal interactions are responsible for signalling the events starting with tooth initiation and leading to differentiation of the odontoblasts and ameloblasts responsible for dentin and enamel secretion respectively. These interactions determine the early morphogenetic events in tooth development[23] as well as the later processes that give rise to odontoblast[24] and ameloblast[25] differentiation. Growth factors are the diffusible cell-signalling molecules that pass between the epithelial and mesenchymal compartments of the tooth germ and are responsible for these interactions. Comparison of the later processes of tooth development responsible for odontoblast differentiation with the events of reparative dentinogenesis and dentin bridge formation may provide valuable insights into the molecular control of dental repair.

During the late bell stage of tooth development, the inner dental epithelium and its associated basement membrane signal the peripheral cells of the dental papilla to differentiate into odontoblasts. Growth factors, particularly of the Transforming Growth Factor-β (TGF-β) family, appear to be important molecules mediating this signalling of odontoblast differentiation.[24,26,27] Secretion of these growth factors by the inner dental epithelium and their sequestration within the dental basement membrane for presentation to the dental papilla cells for signalling of odontoblast differentiation (Figure 5) provide the temporo-spatial control of these processes in the tooth germ. However, in the mature tooth, similar processes must occur to allow differentiation of a new generation of odontoblast-like cells for dentin bridge formation. In the absence of dental epithelium in such reparative situations, it has been necessary to look for alternative signalling mechanisms.

Dentin chips arising from operative debris have long been known to be auto-inductive for reparative dentinogenesis.[28] Experimentally, demineralized dentin matrix and isolated dentin matrix components are also capable of inducing reparative dentinogenesis and bridge formation at sites of pulp exposure,[29-33] as well as ectopic bone formation.[34] This implies that dentin matrix contains bioactive components and is not as inert as sometimes presumed. The collagenous and non-collagenous proteins of dentin have been well reviewed,[35-37] but the emphasis has been on the quantitatively more important components. It has now been possible to identify a number of growth factors in dentin matrix, which, while quantitatively minor

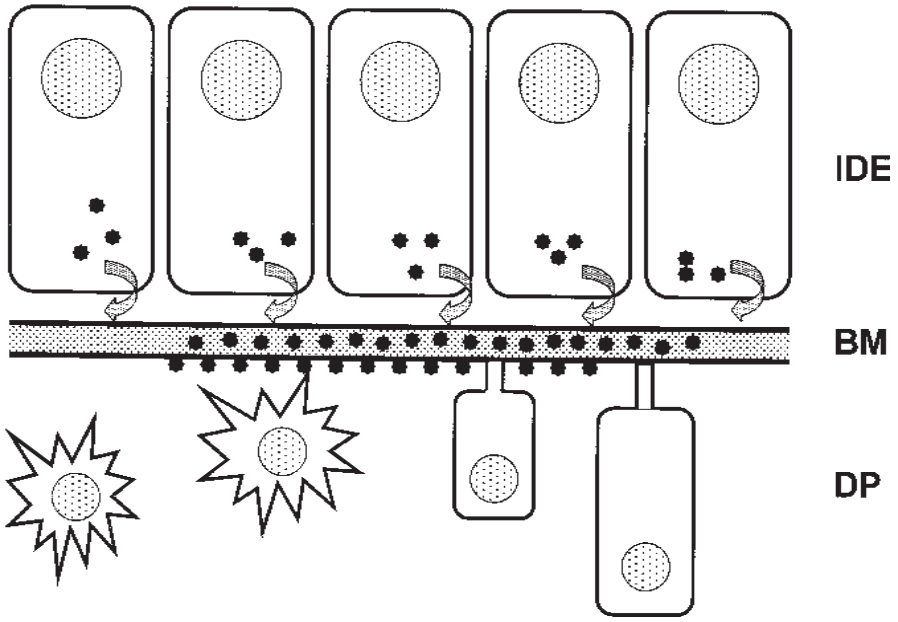


Figure 5. Growth factor (small black stars) secretion by the inner dental epithelium (IDE) during the late bell stage of tooth development leads to sequestration and immobilization of these growth factors in the dental basement membrane (BM) for temporo-spatial presentation to the cells of the dental papilla (DP) and signalling of odontoblast differentiation.

components, may have potent biological effects. Once incorporated within dentin matrix, these growth factors become "fossilized" and retain their biological activity through the protection offered by their interaction with dentin extracellular matrix components. A key family of growth factors, which have been identified in dentin, are members of the TGF-β family of growth factors.[38,39] Members of this growth factor family have been implicated in signalling odontoblast differentiation during tooth development[26,27] and, thus, may be important signalling molecules during repair. Other growth factors have also been identified.[38,40] Dentin matrix therefore contains a cocktail of bioactive molecules with potent cell signalling properties, which may be released into the pulp environment during tissue injury. These growth factors are contained within both the soluble and insoluble tissue compartments of the matrix, and their release or exposure may therefore vary under different tissue conditions.

The origin of these growth factors in dentin matrix is probably largely the odontoblast cell,[41] and after secretion, they interact with extracellular matrix or mineral components of the dentin thereby becoming incorporated or sequestrated within the matrix.[42,43] The specificity of the interaction with matrix components may be quite precise since, in the human tooth, odontoblasts express all three isoforms of TGF (TGF-1, 2, and 3) but only TGF-β1 becomes sequestrated within the matrix.[39] It appears that only TGF-1 is capable of interacting with the proteoglycans of dentin matrix, decorin, and biglycan, and thus the other isoforms show far less affinity for the matrix (Baker, Sloan, and Smith; unpublished observations). These interactions with matrix components may be very important in protecting the biological activity of growth factors since their half-life may be only of the order of a few minutes when they exist in the free state in serum.[44]

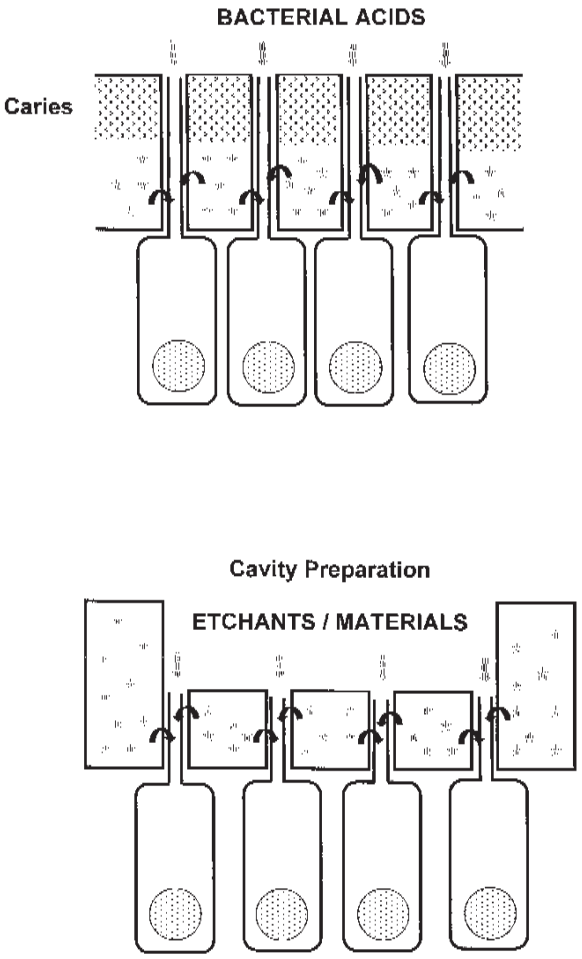
The potential functional activities of the growth factors sequestrated within dentin matrix may be quite extensive. In addition to the induction of odontoblast-like cell differentiation alluded to above, these molecules may signal cell proliferation and migration within the pulp. The presence of angiogenic growth factors in dentin matrix may be important in stimulating new capillary formation at sites of repair,[40] and TGF-βs may help to dampen the inflammatory response in the pulp.[45] It must be recognized, however, that the cells are being potentially exposed to a cocktail of growth factors and that the presence of more than one growth factor at any time in the

environment of the cell may lead to complex interactions and cell responses. Such responses may not be easily predictable based on the summation of the known activities of the individual growth factors. Furthermore, their release during dental injury is likely to be variable and rather uncontrolled and, hence, to contribute to the unpredictability of their overall effects. The presence of bacterial irritation and pulpal inflammation in carious pulp exposures may also modify growth factor release and activity during repair.[19,20] In the light of this, it is perhaps not surprising that clinical management of dental disease is not always as predictable as desired, even under ideal conditions.

Whilst the sequestration of growth factors within dentin matrix provides one possible pool of these cell-signalling molecules in the injury situation, fibroblasts and other pulp cells may be other sources. Both the physiological cell populations of the pulp and the inflammatory cells, which locally infiltrate the tissue at sites of injury, also express a number of growth factors and may contribute to the overall tissue response.[41] Although these sources of growth factors may not become "fossilized" in the same way as those deriving from the dentin matrix because of the greater turnover in the extracellular matrix of the pulp soft tissue, they will nevertheless contribute to the more immediate cellular responses. Thus, the overall response in the pulp is likely to be a summation of the effects arising from release of growth factors from the dentin matrix and more local secretion from the cells in the pulp itself.

Release of Growth Factors from Dental Tissues and Modulation of Expression During Injury and Repair

Growth factors may be released from the dentin matrix as a result of both injury events to the tissues and clinical restorative procedures. During caries, diffusion of acidic plaque bacterial metabolites into the tissue will lead to demineralization and release of soluble extracellular matrix components, including growth factors (Figure 6). While some outward diffusion may occur, many of the released components will diffuse in a pulpal direction where they interact with the cells. The ongoing nature of the carious demineralization process will probably result in release of these growth factors over a prolonged period of time, thereby facilitating opportunities for cell signalling. The demineralization process will also unmask or expose the insoluble dentine matrix. While this insoluble matrix will predominantly be made of collagen, pools of growth factors are also associated with the insoluble matrix.[39] Un-



Top: Caries attack of the tooth results in bacterial acids diffusing along the dentinal tubules and solubilizing and exposing matrix-bound growth factors during the demineralization process.

Bottom: During cavity preparation, application of etchants as well as subsequent restoration with some materials may solubilize and expose matrix-bound growth factors.

Figure 6. Mechanisms of release of growth factors (grey stars) from the dentin matrix and their subsequent diffusion along the dentinal tubules to interact with the odontoblasts and pulp cells

masking of these growth factors immobilized in the dentin matrix may be important in their presentation to pulp cells for signalling events associated with defense and repair response. With caries lesion progression, the initial diffusion of acid into the tissue will be followed by diffusion of plaque bacteria-derived proteolytic enzymes, which will degrade the insoluble dentin matrix. Release of the associated growth factors will further supplement the soluble pools of growth factors from the tissue. Thus, the degradative events of caries may be important in releasing and unmasking bioactive molecules, which have the potential to signal many of the defense and repair events in the tooth. The endogenous proteolytic enzymes (Matrix MetalloProteinases, MMPs) present in dentin matrix[46] might also participate in releasing and unmasking bioactive molecules, even in the absence of bacteria, if their normal regulatory control becomes compromised in pathologic conditions.

Growth factors may also be released from the dentin matrix as a result of clinical restorative procedures as well as during injury. Cavity etchants and other tissue treatments are widely used in restorative dentistry and endodontics to provide a suitable surface for adhesion of materials. Many etchants are based on either acids or EDTA, which have demineralizing properties and will act to release soluble growth factors and unmask insoluble growth factors[47] from the dentin matrix in the same way as bacterial acids during caries (Figure 6). Thus, these agents may have a biological as well as a physico-chemical basis to their action. It should be appreciated, however, that studies on the release and unmasking of growth factors from the dentin matrix during cavity preparation have been performed *in vitro,* and establishment of proof of such concepts in the clinical situation is awaited. The influence of growth factors on biological events may be of greatest importance in deeper cavity preparations where repair, seen as reactionary dentin secretion, seems to be greater.[48]

Although leaching of components from some restorative materials may be seen after application to cavity preparations, most will not exhibit demineralizing properties on the dental tissues. Calcium hydroxide is one of the most commonly used pulp capping agents, and although it is not acidic, it does have some solubilizing effect on dentin matrix.[49] Amongst the components of dentin matrix solubilized by calcium hydroxide is the growth factor TGF-1, the release of which may contribute to the initiation of dentin bridge formation following pulp capping with this agent. Thus, there may be a more rational biological explanation for the action of calcium hydroxide rather than the more traditional view of it "irritating" the pulp cells and stimulating odontoblast-like cell differentiation for dentin bridge formation.[50]

Our traditional views of a) dentin being a relatively inert substrate and b) clinical restorative procedures having a predominantly physico-chemical basis must therefore be brought into question. The effects of both the initial caries injury and its subsequent clinical treatment may have profound effects on the subsequent course of cellular events in the dentin-pulp complex, and attention to these points has considerable potential for preservation of pulp vitality and patient management.

Growth Factors as Mediators of Repair and Regeneration in the Dentin-Pulp Complex

The processes of reactionary and reparative dentinogenesis (Figure 2) at sites of dental injury are responsible for secretion of tertiary dentin matrices, which either increase the dentin barrier between the site of injury and the underlying cells in the unexposed pulp or provide a dentin bridge across the exposed pulp. Growth factors may be key molecules in the signalling of the biological events responsible for these processes.

A number of reports of the *in vivo*[51-57] or *in vitro*[58] placement of exogenous growth factors, particularly TGF-βs and Bone Morphogenetic Proteins (BMPs), on exposed pulps have demonstrated the potential of these molecules to signal reparative dentinogenic events. Transdentinal or direct application of TGF-1 and BMP-7 to the odontoblasts of unexposed pulps in cultured tooth slices has also shown the ability of these growth factors to signal reactionary dentinogenesis.[58-60] In addition, application of soluble isolated dentin matrix extracts, containing endogenous growth factors, in unexposed[18,61] or exposed cavity preparations[33] have corroborated these findings. These findings suggest exciting opportunities for biologically based therapeutic approaches to dental tissue repair as well as providing valuable insights into how natural regenerative processes may be operating in the tooth.

To exploit these findings, it is important to appreciate the nature of the signalling processes mediated by growth factors. For reparative dentinogenesis, the initial event will be recruitment of progenitor or stem cells for subsequent differentiation of a new generation of odontoblast-like cells to replace those that died as a result of the injury. While a specific dental stem cell population has been suggested,[62,63] a variety of derivations for progenitor cell populations in the pulp may exist.[9,64] Growth factors of the TGF-β family, as well as various dentin matrix components, appear to be chemotactic for mesenchymal cells, and the migration of these cells to sites of injury is unlikely to pose problems. Induction of cytodifferentiation of odontoblast-like cells from these progenitor cells appears to be a key signalling role for growth factors. Application of various growth factors (TGFs, BMPs, and Insulin Growth Factor, IGF) to cultured dental papillae isolated from tooth germs[26,27] and of TGF-3 to needle punch injuries in the odontoblast area of cultured tooth slices[58] has been shown to induce odontoblast-like cell differentiation. Interestingly, however, this signalling appears to be able to proceed with several different growth factors raising questions about the specificity of the signalling process. In dental papilla isolated from tooth germs, only TGFs were able to reproduce the normal gradients of odontoblast differentiation and morphology of the tissues.[26,27] During application of growth factors to exposed pulps in capping situations, several growth factors stimulated reparative responses,[51-57] but the reparative dentin matrix secreted was of variable structure ranging from a tubular matrix resembling physiological dentin to atubular, osteodentin-like matrices. Similar variations in the structure of reparative dentin matrices are not unusual in dentin bridges, which form as a result of natural regenerative processes. It is possible that odontoblast-like cells represent a spectrum of cell phenotypes depending on the progenitor cells from which they are derived, the signalling events responsible for their differentiation, and other factors such as the regulation of these signalling events. Other factors, such as bacterial irritation and pulpal inflammation, may also exacerbate the heterogeneity observed during reparative dentinogenesis. The absence of reparative dentinogenesis in ferret teeth exhibiting reversible pulpitis after BMP-7 implantation or gene transfer,[19,20] despite the inductive effects of BMP-7 implantation on reparative dentinogenesis in healthy teeth,[51,52] highlights the complexity of cellular events under such conditions.

Despite the possible heterogeneity in phenotype of odontoblast-like cells, there seem to be a number of parallels in the processes responsible for signalling of the differentiation of these cells and physiological odontoblast differentiation during tooth development. The exposure of matrix-bound growth factors in the dentin matrix potentially provides an immobilized signal for presentation to pulp stem cells for induction of differentiation of odontoblast-like cells in a manner similar to the dental basement membrane presentation of enamel organ derived growth factors during embryonic odontoblast differentiation. An essential difference between these two processes, however, is that during development the growth factor signal is epithelially derived while, during repair, it arises from secretion by the primary odontoblasts.

Following cytodifferentiation, stimulation or up-regulation of the secretory activity of the odontoblast-like cells is required for deposition of the reparative dentin matrix and dentin bridge formation (Figure 2). This event is also common to reactionary dentinogenesis where the secretory activity of the surviving odontoblasts beneath the injury is up-regulated. Again, growth factors may be key to the signalling of this up-regulation of secretory activity since TGFs and BMPs have been demonstrated to elicit such effects when applied to odontoblasts in cultured tooth slices.[58,60] Such effects concur with the known actions of these growth factors on a variety of cell types in the body. However, it raises interesting questions as to the nature of the control of odontoblast secretion in the tooth. During physiological tooth development, there is close regulation of dentin secretion giving rise to teeth of remarkably reproducible crown and root size and shape. Following completion of root formation, there is an abrupt decline in odontoblast activity and the subsequent maintenance of these cells in a near quiescent state. It is unclear what regulates this abrupt transition in odontoblast activity in terms of dentin secretion, but clearly it may mirror what controls the up-regulation of odontoblast secretion during repair. Thus, growth factors may regulate secretory activity throughout the odontoblast life cycle, possibly through autocrine control (Figure 3). Elucidation of these molecular control mechanisms may provide valuable tools for switching on and off dentin secretion for therapeutic purposes.

Clearly, close regulation of the signalling processes mediated by growth factors is required to elicit the overall control of reparative events following tissue injury in the tooth. Opportunities for release of

growth factors from the dentin matrix as well as their production by both pulp and inflammatory cells may lead to rather uncontrolled reparative events and provide some explanation for the variety of pulp responses seen following dental injury.

Therapeutic Approaches to Dental Tissue Repair and Natural Regeneration as a Blueprint for Tissue Engineering

The experimental induction of reactionary and reparative dentinogenesis and dentin bridge formation with growth factors offers exciting opportunities for novel therapeutic approaches to dental tissue repair. Initially, attention to the natural regenerative processes, which may be stimulated, could provide valuable clinical benefits during restorative dentistry. Thus, consideration of factors such as residual dentin thickness and etching during cavity preparation may optimize the natural regenerative processes. Application of calcium hydroxide in the base of cavities, where appropriate, may also stimulate cellular repair.

While no pulp-capping agents based on growth factors are presently commercially available, their introduction is likely to be imminent. The delivery mechanisms for such agents may be varied, although syringe or paste application might prove appropriate. The rather variable nature of the reparative dentin matrices they can stimulate may be usefully harnessed for different clinical applications. Thus, stimulation of a physiological-like tubular dentin structure may find application in restoration of lesions of the crown, while an atubular, bone-like matrix might prove valuable as an alternative approach in root canal therapy where an impermeable barrier is required. The great benefit of these biological approaches to dental tissue restoration over many conventional dental materials is that the reparative matrices become an integral part of the tooth, overcoming many of the problems of retention of a restoration and possible marginal bacterial microleakage. Some existing materials, such as calcium hydroxide[50] and mineral trioxide aggregate,[65] can also promote reparative dentinogenesis and dentin bridge formation. With these materials, an osteotypic form of matrix tends to be initially deposited upon which a more tubular matrix resembling dentin is subsequently secreted. The cellular events leading to dentin bridge formation with these materials may resemble those seen with growth factor-mediated approaches. However, therapies directly based on growth factors may offer advantages over these more traditional materials through greater control of the biological effects of these molecules and targeting of their action.

Importantly, these biological approaches to dental tissue regeneration and repair start to provide us with a blueprint for developing exciting tissue engineering solutions for the dental tissues. While engineering of the entire tooth represents our ultimate goal, this is clearly going to provide significant challenges. Nevertheless, in the shorter term, tissue engineering of the pulp may be more easily achieved[66] and be of immense clinical benefit, especially in the pulpotomy situation. Traditional tissue engineering approaches of seeding cells onto structural scaffolds will require careful consideration for the pulp where the pulp extracellular matrix has a characteristic gelatinous consistency and is probably important in controlling cell behavior. The role of growth factors as components of the matrix/scaffold is likely to be critical in providing the necessary regulation of cell behavior to ensure physiological-like function. In combination with growth factor-based treatment modalities directed at reparative dentinogenesis and dentin bridge formation, such tissue engineering approaches may provide very effective clinical tools. Development of tissue engineering strategies that exploit the cell-signalling properties of growth factors have the potential for profoundly changing the clinical management of dental disease and the restoration of the dentin-pulp complex.

Acknowledgments

This review is dedicated to the memory of Jean-Victor Ruch from Strasbourg, France, with whom many stimulating discussions have provided much inspiration for research on the cellular control of tooth repair. I am grateful for the contributions of my colleagues and collaborators and other researchers in the field, which have allowed an insight into the biological processes involved in tooth repair. I gratefully acknowledge the support of the Wellcome Trust and UK Medical and Engineering & Physical Sciences Research Councils for generously providing research funding.

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