

Preserving the Vital Pulp in Operative Dentistry: I. A Biological Approach

ANTHONY J. SMITH, PETER E. MURRAY AND PHILIP J. LUMLEY

Abstract: This is the first in a series of four papers aimed at understanding human pulpal responses to tissue injury, cavity preparation and restorative events. This article provides an insight into the exquisite regenerative potential of the dentine–pulp complex which underpins the success of restorative dentistry.

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Clinical Relevance: Appreciation of the natural regenerative properties of the dentine–pulp complex provides the clinician with a basis for treatment planning to achieve successful vital pulp therapy.

Preservation of pulp viability continues to be a major challenge for restorative dentistry in view of the numbers of new and replacement restorations performed each year. It has been estimated that some 290 million restorations are placed annually in the USA, and some 16 million permanent restorations are placed per year by NHS dentists in England and Wales. The proportion of the population aged over 65 years is expected to double in the next 20 years; elderly people are increasingly retaining their natural teeth and require dental treatment into old age. More than ever before, it is important to consider measures to preserve the vitality of teeth, avoiding complications and increasing treatment success and longevity. The sizeable

difference between the success of cavity restorations that preserve pulp vitality and those which injure pulp vitality means that it is worth concentrating efforts on preserving the vital pulp following treatment. The success of short-term treatment outcomes following pulp exposure injury is in the range of 44–97%, while treatment outcomes in the absence of pulp injury can approach 100% (the success rate varies with materials and conditions).

The exquisite regenerative potential of the dentine–pulp complex underpins the success of much of restorative dentistry. However, the relative influence of the various restorative events on pulpal viability and regeneration has long been somewhat empirical. An appreciation of the underlying biological processes taking place in the dentine–pulp complex during injury and repair, and how restorative events can modify them, offers considerable potential for exploiting the regenerative potential of these tissues.

INJURY TO THE DENTINE–PULP COMPLEX

The intensity and duration of injury to the tooth, whether it be of carious, traumatic or other origin, has considerable implications for subsequent pulpal responses. During mild grades of injury, such as those involving peripheral caries lesions and mild forms of erosion, the odontoblasts and other pulpal cells underlying the lesion may well survive, and are stimulated or upregulated to secrete a *reactionary type* of tertiary dentine matrix (Figure 1). This is essentially a wound-healing response to reconstitute circumpulpal dentine as a response to injury. However, injury of greater intensity, such as that involving pulp exposure or progressing caries, may kill the odontoblasts and other pulpal cells beneath the lesion. Often, these cells will be replaced by a new generation of odontoblast-like cells, secreting a *reparative type* of tertiary dentine. If the pulp is exposed, this reparative dentine may lead to bridge formation, contributing to restoration of tissue integrity.

Although the outcomes of these two injury situations are similar in respect of deposition of a tertiary dentine matrix at the pulp–dentine interface, appreciable differences exist in the complexity of the biological events taking place and the opportunities for failure to achieve regeneration. Reactionary dentinogenesis simply requires stimulation of existing odontoblast cells, whilst reparative dentinogenesis needs recruitment of progenitor cells from the pulpal

Anthony J. Smith, BSc, PhD, Professor of Oral Biology, **Peter E. Murray**, BSc, PhD, Research Fellow in Oral Biology, and **Philip J. Lumley**, PhD, BDS, MDentSci, FDS RCPS, Senior lecturer in Restorative Dentistry, School of Dentistry, University of Birmingham.

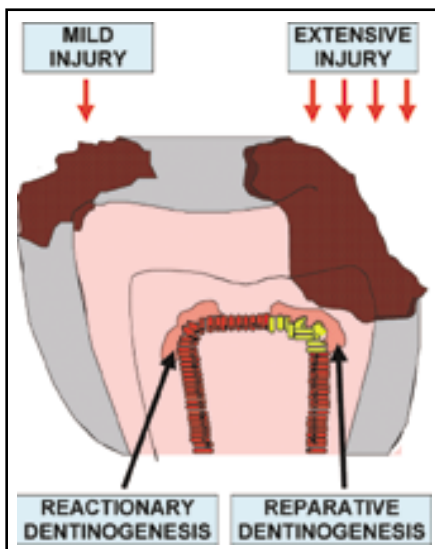


Figure 1. Schematic diagram of the reactionary and reparative variants of tertiary dentinogenesis as responses to tissue injury of increasing magnitude.

population, their differentiation into odontoblast-like cells and then stimulation of matrix secretion (Figure 2). Such a complex chain of biological events provides greater opportunity for failure.

Other tissue events may exacerbate the situation. For instance, the inflammation accompanying any injury is primarily a defensive mechanism, but if uncontrolled, may lead to widespread tissue damage and severely compromise regeneration; bacterial microleakage following cavity restoration will almost certainly perpetuate and exacerbate pulpal inflammation.

Injury to the pulp may become more intense as a result of cavity preparation and restoration. Drill speed, coolant and operator pressure can all influence pulpal responses. The extent of cavity preparation and unnecessary iatrogenic removal of dentine will both adversely affect pulpal cell survival and repair.

Use of etchants during cavity preparation can facilitate adhesion of materials, but will also potentially influence pulpal cell responses. Choice of restorative material, whilst of lesser direct importance than these other factors, can also affect pulpal cell

survival and repair. This is especially true in respect of the ability of a material to prevent bacterial microleakage. The influences of these various factors are discussed in the succeeding three papers in this series.

KEY BIOLOGICAL EVENTS IN PULPAL REPAIR

The two key events in pulpal repair are:

- progenitor cell recruitment; and
- odontoblast-like cell differentiation and stimulation of dentine matrix secretion.

Progenitor or stem cell recruitment will only be necessary during reparative dentinogenesis, but represents a critical step in the repair process. The origin of these progenitor cells has long remained obscure: cells within the cell-rich layer of Höhl immediately underlying the odontoblasts have been favourite candidates,¹ although other pulpal cell populations have also been implicated including pericytes from the vasculature. It is possible that the relative importance of these populations may vary with age and that the decline in sub-odontoblastic Höhl cells with ageing may mean that pericytes or other progenitor cells assume greater importance. Although this area remains controversial, recent advances in stem cell biology may soon advance our understanding. This could be critical to care and management of the ageing patient, in whom regenerative capacity of the tissues may be compromised.

Both the induction of odontoblast-like cell differentiation during reparative dentinogenesis and stimulation of dentine matrix secretion (reactionary and reparative dentinogenesis) require initiation by molecular signalling. This is an area in which significant advances have been made recently. Study of these signalling mechanisms during tooth development has been invaluable in highlighting how developmental events may occur during repair.

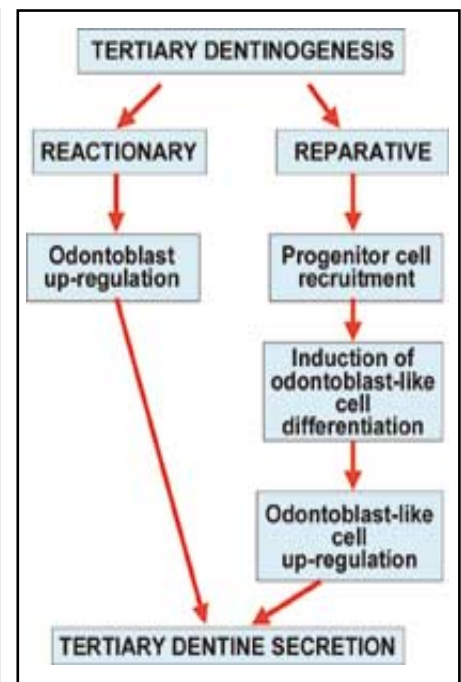


Figure 2. Comparison of the biological processes involved during reactionary and reparative dentinogenesis leading to secretion of tertiary dentine.

TOOTH REPAIR RECAPITULATES DEVELOPMENTAL EVENTS

Epithelial-mesenchymal interactions control organ development throughout the body. The inner enamel epithelium and its associated basement membrane in the enamel organ are responsible for signalling induction of odontoblast differentiation in the dental papilla during development.² The molecular signal for this induction of odontoblast differentiation and the subsequent stimulation of matrix secretion by these cells is a class of molecules called *growth factors*. These are small protein molecules with very potent bioactive effects on a variety of cellular activities and behaviours including cell division, differentiation, migration, etc. They are found throughout the body and are responsible for regulation of most cellular events. One sub-class of these molecules, the Transforming Growth Factor- β (TGF- β) family,³ appears to be responsible for signalling odontoblast differentiation during tooth development. TGF- β molecules are secreted by the inner enamel

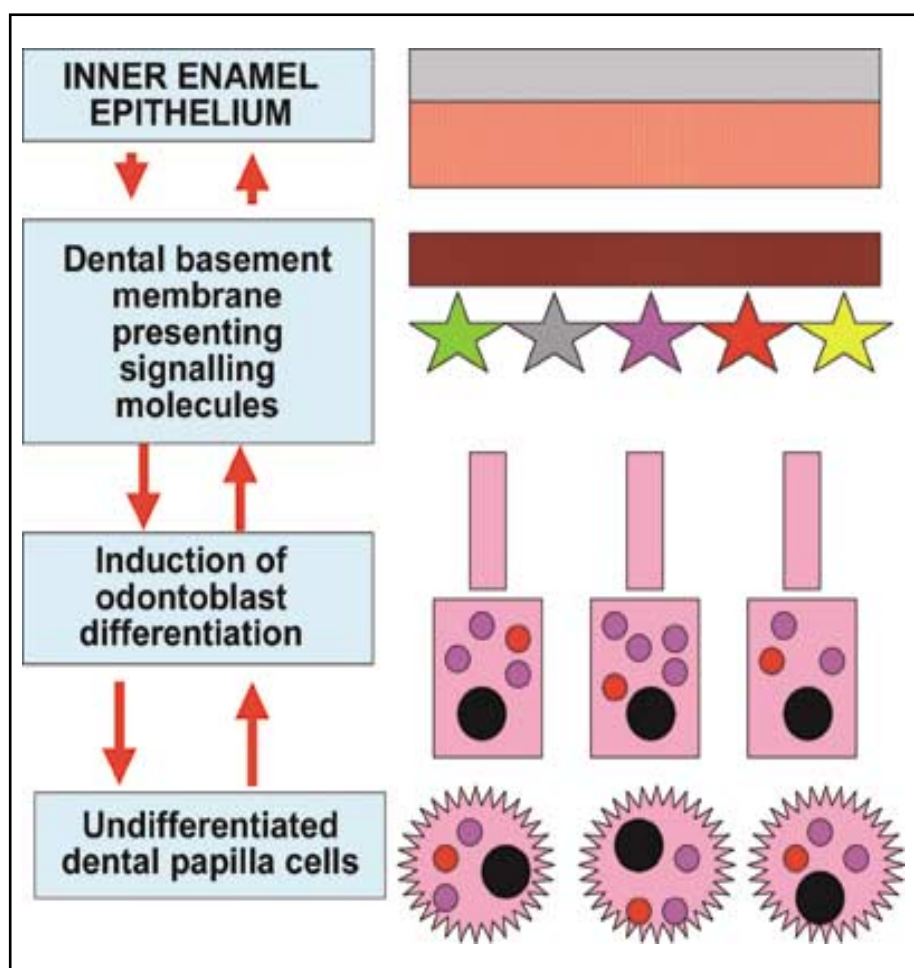


Figure 3. Schematic diagram of the temporospatial presentation of TGF- β signalling molecules in the dental basement membrane for induction of odontoblast differentiation during tooth development.

epithelial cells and become associated with the dental basement membrane, where they are temporospatially presented to induce the peripheral cells of the dental papilla to differentiate into odontoblasts and then secrete the primary dentine matrix (Figure 3).

Once differentiated, odontoblasts synthesize and secrete TGF- β s as well as a number of other growth factors, including ones that stimulate angiogenesis, and deposit them within the dentine matrix.^{4,5} Thus, contrary to popular belief, dentine matrix is not an inert, predominantly inorganic material, but a tissue containing a variety of bioactive molecules.

If released during injury or cavity restoration, these growth factors have the potential to influence pulpal events dramatically by signalling odontoblast-like cell differentiation and stimulating

the odontoblasts to secrete tertiary dentine.⁶ Reparative events in the dentine–pulp complex thus tend to recapitulate or mimic developmental events. It is therefore critical that we develop an understanding of how injury to the tooth and subsequent cavity restoration may influence the release of these growth factors with their potent cell-signalling effects.

DENTINE MATRIX DISSOLUTION AND GROWTH FACTOR RELEASE

During caries, plaque bacterial acids diffuse through the dental tissues and dissolve the enamel and dentine matrix. Although this matrix dissolution is commonly thought of as a simple demineralization process, a number of

soluble components of the dentine organic matrix associated with the mineral crystals will also be dissolved and potentially diffuse along the dentinal tubules in a pulpal direction (Figure 4). The odontoblasts and pulpal cells, in addition to being exposed to the bacterial acids, may thus also come into contact with dissolved matrix components. These will include growth factors, which are associated with both the soluble and insoluble tissue components of the dentine matrix.

During cavity preparation, conditioning of the cavity walls is frequently undertaken to improve adhesion of materials to the dentine. The etching agents used include a variety of acid-based chemicals, which are capable of dissolving the smear layer and debris produced as a result of cavity cutting (the primary aim of these agents is to dissolve this tissue debris and etch the cavity walls before placement of the restoration). There is therefore scope for dissolution of any matrix components in the debris or on the cavity surfaces, which may diffuse down the dentinal tubules in a pulpal direction. Any growth factors dissolved from the matrix in this way might act as signalling molecules to the odontoblasts and other pulpal cells.

In vitro dissolution of powdered dentine matrix has demonstrated that most etching agents are capable of releasing TGF- β 1 and other growth factors.⁷ The effectiveness of these agents to dissolve TGF- β 1 was in the rank order of EDTA > Phosphoric acid \geq Citric acid \gg Polyacrylic acid > Nitric acid. Because growth factors like the TGF- β s are found in both the soluble and insoluble tissue compartments of the dentine matrix, there is scope for both their release from the soluble compartment and subsequent pulpal diffusion and their exposure on the surface of the insoluble matrix. The latter mechanism may be important in pulpal exposure, where TGF- β s may signal odontoblast-like cell differentiation for reparative dentinogenesis in a manner analogous to that during tooth development. Staining for TGF- β 1 after etching of a cavity preparation reveals

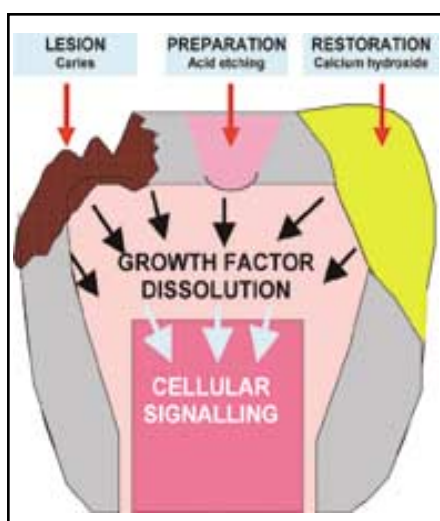


Figure 4. Schematic diagram of dissolution of growth factors and other matrix components from dentine during tissue injury, cavity preparation and restoration.

exposure of these growth factors on the cavity walls⁸ (Figure 5), where they may elicit cell signalling effects. Consideration should therefore be given to the action of these agents in releasing bioactive molecules from the matrix as well as their effects on facilitating adhesion of materials.

TGF- β 1, and possibly other growth factors, may also be released by the action of components leaching from restorative materials. Despite its high pH, calcium hydroxide has a slight demineralizing action on dentine, and in the process releases TGF- β 1 from the matrix. This may help to explain the beneficial effects of calcium hydroxide on repair in the dentine-pulp complex. It will be interesting to learn whether any other restorative materials have similar effects. Thus opportunities exist to release growth factors from the dentine matrix at the stages of initial injury to the tissue, during cavity preparation and during cavity restoration (Figure 4). Release of these potent cell-signalling molecules may have important implications for reparative events in the dentine-pulp complex.

Development of novel restorative materials containing growth factors represents another potential future approach, but a number of problems of delivery and regulation of the repair

response will need to be addressed before such approaches can be considered. Harnessing the natural regenerative properties of the dentine-pulp complex remains the most fruitful approach for the present.

EFFECTS OF DENTINE MATRIX COMPONENTS ON REPAIR

Appreciation of the bioactive properties of dentine matrix components has led to experimental attempts to investigate their effects on repair processes in the dentine-pulp complex. Implantation of demineralized dentine matrix or isolated preparations of dentine matrix components in cavities prepared in teeth of experimental animals stimulated a tertiary dentinogenic response.^{9,10} This helps to explain the induction of dentinogenesis, which has often been reported around dentine chips or operative debris pushed into the periphery of the pulp during cavity preparation. When the isolated preparations of dentine matrix components were purified to enrich their growth factor content before implantation, the tertiary dentinogenic response was magnified. Recently, pure growth factors have been implanted in experimental cavities and have yielded similar responses. The potential action of growth factors, particularly of the TGF- β family, to signal tertiary dentinogenesis provides us with an understanding of

the biological basis of a response that has been recognized for more than a century, namely the ability of the tooth to deposit a matrix of repair.

RESTORATIVE EVENTS AND PULPAL RESPONSES

The succeeding three papers in this series describe the relationships between restorative events and the pulpal responses. It is important to recognize that these all have a strong biological basis.

The depth and extent of cavity preparation have repercussions for the degree of injury to the odontoblasts and their potential survival. Whilst shallow cavity preparation will frequently lead to cutting of the odontoblast process, this often appears to have the capacity to self-heal. However, deeper cavity preparation can cause greater injury from which the odontoblast may not recover, and the cell could die. The pulpal response to this will be a more complex, regenerative one.

Patency of the dentinal tubules confers the property of permeability on the dentine matrix. Although a pulsatile, positive pressure exists within the pulp, it does appear that components can diffuse down the tubules against this gradient in a pulward direction (how far different components can diffuse down the tubules is unclear). This may be important in terms of cytotoxic components leaching from materials

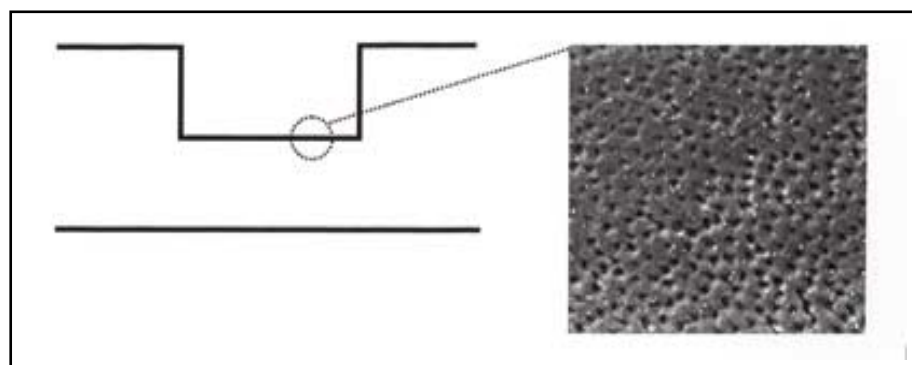


Figure 5. Scanning electron micrograph of an etched cavity preparation (diagram shows area of examination) immunostained for TGF- β 1. Exposure of TGF- β 1 molecules in the matrix can be seen by the bright white dots showing the binding of the gold-labelled antibodies.

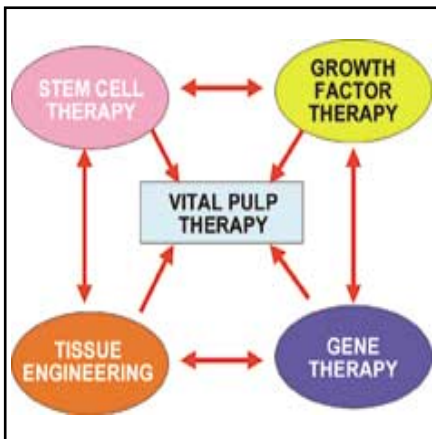


Figure 6. Future possible biological approaches to vital pulp therapy.

and reaching the pulpal cells, or bioactive signalling molecules (like growth factors) being released from the matrix and diffusing to interact with surface receptors on the odontoblasts and other pulpal cells.

Inflammation represents a defence reaction by the pulp after injury and restoration. However, if uncontrolled, inflammation may lead to excessive tissue damage and ultimately pulp necrosis.

Clearly, factors controlling the pulpal responses to restorative events are multi-factorial and empirical approaches to understanding their relative importance will provide limited benefit. Quantification of individual factors in the restorative process will be the key to unravelling how these complex events interact to produce the final outcome. In this way, it may be possible to predict how various treatment strategies can be exploited to provide optimal outcomes.

FUTURE BIOLOGICAL APPROACHES

Recent advances in pulp biology have provided us with an understanding of the biological processes underlying natural regenerative events following injury to the tooth and how we might use them to optimize treatment outcomes. Advances in medicine are opening up a variety of exciting potential new treatment strategies for

tissue injury, some of which may have future application to vital pulp therapy (Figure 6).

Stem cell therapy provides one of the most exciting future potential approaches to vital pulp therapy. Preservation of an adequate stem or progenitor cell population for repair is one of the more important problems for cases of more extensive pulpal injury. If we can introduce a stem cell population capable of eliciting a specific dentinogenic response into the pulp, we may be able to consider true biological approaches to the restoration of the severely compromised pulp. Such approaches might also be enhanced by use of synthetic matrices or scaffolds for the cells to provide tissue engineering solutions to vital pulp therapy. Use of growth factors to signal tissue repair processes has already been experimentally shown to be capable of signalling repair of the dentine-pulp complex. Gene therapy to switch on the expression of these or other signalling molecules also represents a future direction for biological control of dental repair.

Nevertheless, such approaches will also need to consider the regulation of reparative events to ensure that they do not proceed in an uncontrolled manner leading to obliteration of the pulp chamber. Whilst they offer exciting opportunities for developing new future treatment modalities, extensive research will be required before these approaches are clinically viable and it is unlikely that they will be seen in practice for a decade or more. However, harnessing our understanding of the natural regenerative properties of the dentine-pulp complex is an achievable target, and the next 5 years is likely to see much of restorative practice using this approach to optimize the clinical outcomes of treatment.

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JANUARY/FEBRUARY Self-Assessment Answers

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|---------------|---------------|
| 1. B, C, D | 6. A, B, C, D |
| 2. A, B, C, D | 7. A, C, D |
| 3. B, D | 8. A, C |
| 4. A, C, D | 9. A, B, D |
| 5. B, D | 10. B, C |