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REVIEW

Dental regeneration and materials—a partnership

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Abstract Considerable focus on the biocompatibility of dental materials over the last three decades has provided a platform for a wealth of studies on the cellular and molecular responses of the cells of the pulp to injury, both from the disease process and from subsequent restorative intervention. These studies have been fundamental to understanding not only how we can achieve a biocompatible response during restoration of dental disease but also how we can exploit the pulpal cellular responses to achieve wound healing and tissue regeneration in the dentine–pulp complex. This article examines the responses of the pulp to injury and the events leading to tissue regeneration. As new biologically based regenerative therapies emerge for the dental tissues, it is important that these develop in partnership with more traditional approaches using dental materials.

Keywords Dentine · Pulp · Regeneration · Bio-active · Growth factors

Management of carious loss of dentine has long been dependent on restoration with a variety of dental materials. Use of amalgam and other more traditional materials is largely being superseded by adhesive materials with improved aesthetic properties and relatively good performance. Nevertheless, most materials show limitations in restorations with time and 5–10-year survival rates are unsatisfactory [22]. The reasons for failure of restorations are multi-factorial, but the technique sensitivity of placement of modern adhesive systems and increasingly minimal intervention approaches to restorations impose significant challenges to their performance. Nevertheless, the biological responses of the tissues to these restorations have benefited from attention to these points.

Over the last three decades, considerable focus has been placed on the biocompatibility of dental materials (reviewed in [31]) and has led to the development of an international standard [16] to ensure that such biocompatibility is considered in the development of new materials. This has also provided a platform for a wealth of studies investigating the cellular and molecular responses of the cells of the pulp to injury, both from the disease process and from subsequent restorative intervention.

These studies are fundamental to understand how we can explore the cellular responses in the pulp to stimulate wound healing for tissue regeneration. Like any injured tissue in the body, if suitable conditions prevail, the dental pulp has exquisite healing potential and will try to regenerate new dentine and pulp tissue. Dentistry is now at a turning point where research is seeking biological solutions to build on our knowledge of the interaction of materials with the dental tissues to provide cellular- and molecular-based therapies for more effective restoration of diseased dental tissues.

Tissue injury

Caries is a dynamic process and the picture seen clinically at the time of treatment is the summation of all of the phases of the disease, i.e. it is a snapshot in time and provides little insight into the dynamics of the different phases of the disease. While this may be of lesser importance in determining the extent to which tissue excavation will be undertaken, it is critical to understanding

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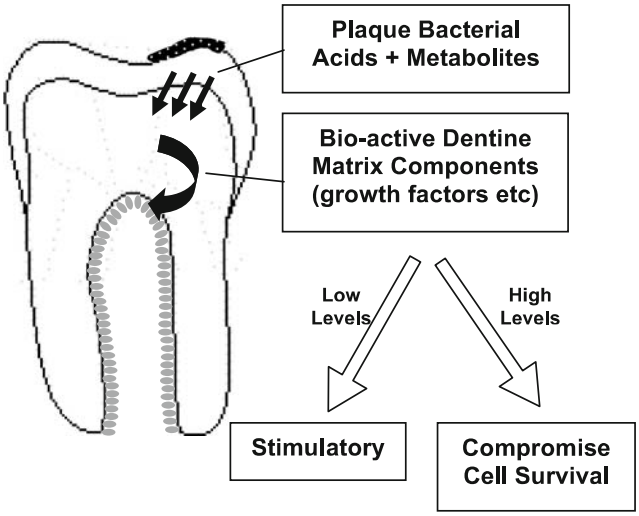

Fig. 1 Schematic diagram of solubilisation of dentine matrix components by plaque bacterial acids and metabolites and their concentration-dependent effects on odontoblast and pulp cell behaviour

how the tissues may respond biologically and the opportunities for tissue regeneration.

A slowly progressing carious lesion of limited penetration of the dental tissues may provide a relatively mild injurious challenge to the cells of the pulp. In contrast, a rapidly progressing deep carious lesion may provide a significant injurious challenge. In the former situation, the cells of the pulp may respond by activation of heat-shock proteins and other molecular responses [18, 19], while the latter situation may lead to local or more extensive cell death in the pulp. Clearly, the latter situation will compromise the vitality of the pulp to a greater extent and constrain its ability to heal itself. This can be readily seen in the regenerative responses of the tissues with stimulation of tertiary dentinogenesis in slowly progressing but not in rapidly progressing carious lesions [5, 6].

Traditionally, it has been assumed that the cellular injury arising during caries is the result of the effects of the plaque bacterial acids and other metabolites diffusing through the enamel and dentine to the pulp. Recent evidence, however, has demonstrated that solubilised dentine matrix components may have dose-dependent effects on pulp cells (Fig. 1). Culture of an immortalised odontoblast-like cell line with lower concentrations of solubilised human dentine matrix components had minimal effect on cell numbers; but, at higher concentrations, these dentine matrix components severely compromised all survival [38]. Interestingly, similar results were observed when the same cell line was cultured in the presence of the growth factor, transforming growth factor (TGF)-β1, at concentrations equivalent to those present in the dentine matrix extracts [12]. This highlights the pleiotrophic effects of this growth factor, which can be inductive for tissue regeneration at lower concentrations and yet can trigger cell death at higher concentrations.

It is clear that the tissue responses to injury can be varied, ranging from regeneration to cell damage and ultimately death. In fact, a combination of these responses may be observed. With a more intense injurious challenge, those odontoblasts and pulp cells immediately beneath a carious lesion may die and, if suitable tissue conditions prevail, a new generation of odontoblast-like cells may differentiate from pulpal stem/progenitor cells.

These new odontoblast-like cells will secrete a tertiary dentine matrix termed reparative dentine [20, 36] (Fig. 2). Such reparative dentinogenesis is responsible for dentine bridge formation at sites of pulp exposure after pulp capping procedures. If the injury is milder in nature, the

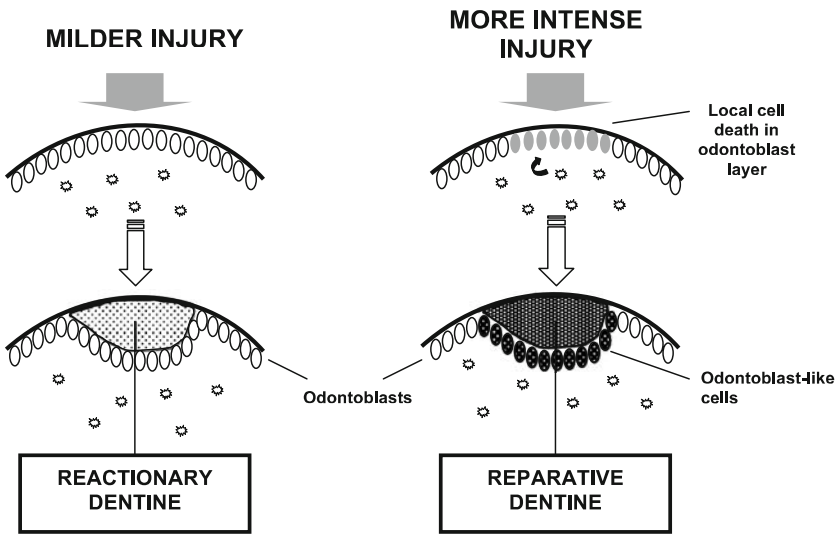

Fig. 2 Schematic diagram of responses to milder and more intense tissue injury. With a milder injury, primary odontoblasts are locally up-regulated and secrete a reactionary dentine matrix. With a more intense injury, local cell death of odontoblasts may occur and, if suitable tissue conditions prevail, a new generation of odontoblast-like cells will differentiate from pulp stem/progenitor cells and secrete a reparative dentine matrix

primary odontoblasts and pulp cells may survive and respond by up-regulating their secretory activity leading to deposition of a reactionary dentine matrix variant of tertiary dentine [20, 36] (Fig. 2). Whilst superficially reactionary and reparative dentines show similarities in that they both lead to matrix deposition at the pulp–dentine interface beneath the site of injury, a marked difference is the presence of tubular continuity for reactionary dentine which is generally absent for reparative dentine. In fact, reparative dentine shows a spectrum of morphology ranging from regular tubularity resembling primary physiological dentine to, in some cases, an atubular appearance.

These differences in regularity and continuity of tubular structure have important implications both for the permeability of the dentine and also for the ability of the formative cells lining its surface (surviving primary odontoblasts or a new generation of odontoblast-like cells) to communicate effectively with deeper areas of the dentine matrix. It is exciting to speculate that, when we fully understand the cellular and molecular events responsible for reactionary and reparative dentinogenesis, we may be able to exploit these processes to develop "designer" therapies whereby a tubular dentine response might be initiated in crown tissues to maintain physiological properties of the tissue and an atubular matrix in endodontic applications to provide an impermeable seal to the apical tissues of the tooth.

In considering pulpal responses to injury, it is also important to remember that cavity preparation may well add to the injury burden for the dental tissues. The depth of cavity preparation will determine to what extent the cellular processes of the odontoblasts are cut, thereby influencing the degree of cell injury. Evidence indicates that in deep cavity preparations with small remaining dentine thickness (RDT), odontoblast survival is compromised and RDT appears to have a powerful influence on the regenerative response of tertiary dentinogenesis [25].

Tissue defence

In the healthy pulp, defence cells comprise a relatively small proportion of the total cell population. Nevertheless, they are important in providing immunosurveillance for the tissue and represent the first line of defence against any injury. Classically, one of the main tissue defence responses that may be observed in histological preparations of carious and injured pulp tissue is inflammation. Whilst this is primarily a defence response, the non-compliant nature of the pulpal environment with its rigid outer shell of mineralised tissue means that sometimes the innate and adaptive inflammatory processes can become uncontrolled. In such situations, inflammation starts to be destructive to the pulp tissue rather than purely defensive and may well compromise pulp vitality (Fig. 3).

A recent comparative study of gene expression in healthy and carious pulp showed that over 400 genes are differentially expressed during caries with many of these being genes involved in inflammatory signalling and which were up-regulated [24]. The complexity of inflammatory events in the pulp during caries is highlighted by the interrelationships between a number of inflammatory mediators and cytokines, especially those of the S100 family [23]. A more robust understanding of the molecular events during pulpal inflammation may provide opportunities for harnessing some of the many novel anti-inflammatory peptides being developed at present within the pharmaceutical industry for more effective control of the inflammatory cascade in pulp during disease.

A key driver of pulpal inflammation is bacterial infection of the tissues. The importance of bacteria and their components in stimulating inflammation is now well established [3, 4, 40] and their presence in the pulp will exacerbate and prolong inflammation [25]. The impact of the inflammatory response on tissue regeneration has been elegantly demonstrated in an animal model of growth-factor-mediated pulp regeneration, where the presence of inflammation was shown to block tissue regeneration [30]. Thus, effective control of inflammation will be critical to development of biological strategies to stimulate tissue regeneration.

With increasing attention to minimal intervention during carious excavation, there is greater risk of not completely removing all infected tissue. Whilst the survival of

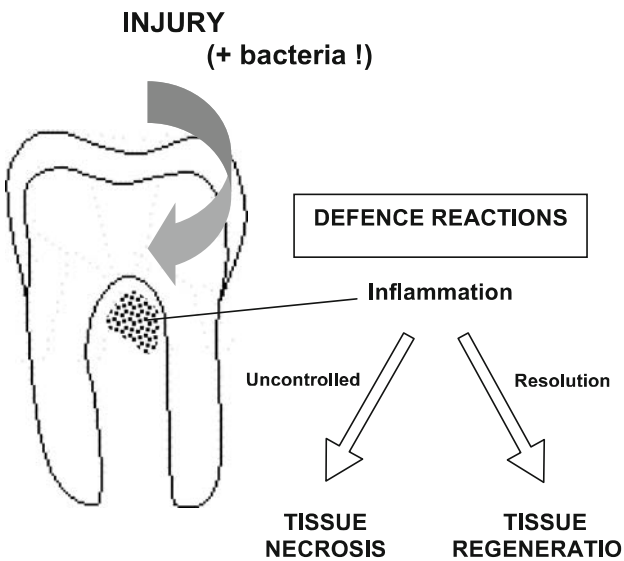

Fig. 3 Schematic diagram of defence reactions in the dentine–pulp complex to tissue injury. Inflammation is the common defence reaction, which as it resolves provides a tissue environment conducive to tissue regeneration. An uncontrolled inflammatory response in the pulp, especially in the presence of bacterial infection, can lead to tissue necrosis

entombed bacteria may be compromised [17], there are opportunities for antibacterial control during restoration. Although there has been limited success in the past using resin composites and dentine bonding systems for antibacterial control (reviewed in [14]), new antibacterial bonding systems [15] provide more effective and selective targeting of bacteria. Control of bacterial infection of the dental tissues and moderation of the inflammatory responses therein will be an important part of providing an environment within the injured dentine–pulp complex conducive for tissue regeneration to take place (Fig. 3).

Vitality of dentine matrix

The structure of dentine, with tubules containing odontoblast processes, has long been recognised. Nevertheless, the importance of this structure to cellular communication within the dentine matrix is often not given adequate consideration. The presence of odontoblast processes together with final lateral processes permeating much of the thickness of dentine means that most of the dentine is in intimate cellular communication with the odontoblasts. Any stimulus to the dentine, whether it be from disease, trauma, surgery or restorative materials, will rapidly trigger a response in the odontoblasts and other pulpal cells. It is therefore crucial to consider dentine as a vital responsive tissue like any other in the body.

Deeper cavity preparation in dentine will compromise odontoblast survival and the residual dentine thickness will have a powerful influence on tissue regeneration through tertiary dentinogenesis in the dentine–pulp complex [25]. Despite the routine use of cavity preparation in restorative dentistry, our understanding of the effects on cellular events of cutting the tissue in this way is still incomplete. During cavity preparation, odontoblast processes running through the dentine matrix will be cut and the ability of the cells to recover from this trauma and the mechanisms responsible still require elucidation. It seems likely that the cellular injury caused by cutting cell processes in shallower cavities may be reversible; but, as the cavity increases in depth, cell recovery is reduced [25]. Such observations provide strong arguments for minimal intervention approaches during excavation of carious dental tissues [17].

The bio-active nature of dentine and tissue regeneration

The organic composition of dentine matrix comprises approximately 90% of collagen with the remaining 10% representing a heterogeneous mixture of a variety of non-collagenous proteins including proteoglycans [10]. Some of the latter proteins may be systemically derived, although most represent secretory products of the odontoblasts during the process of dentinogenesis. Traditionally, dentine has been regarded as a relatively inert substrate, but more recent research has indicated that many of the non-collagenous proteins possess bio-active properties. In particular, dentine matrix contains a cocktail of growth factors, especially those of the TGF-β superfamily [7, 9] as well as various angiogenic growth factors [27]. These growth factors are secreted by the odontoblasts [32] and become sequestered in the dentine matrix, in part because of their interaction with the extracellular matrix components therein [37]. Once sequestrated within the dentine matrix, these growth factors are essentially "fossilised" there and remain protected in their bio-active state until the matrix is degraded during carious or other injurious episodes. Once released from their association with the matrix, these molecules may then diffuse to the cells of the pulp and are available to participate in the regenerative events following tissue injury.

Isolated preparations of dentine matrix components are capable of inducing both reactionary [35, 36] and reparative [33] dentinogenesis; in vivo and in vitro studies have shown that they can induce odontoblast-like cell behaviour in pulp stem cells [21]. Thus, the release of these growth factors from dentine matrix during carious dissolution may be the key to the regenerative responses observed. The mechanisms underlying these responses may represent recapitulation of developmental events where growth factor signalling from the inner dental epithelium, mediated through the dental basement membrane, induces functional differentiation of odontoblasts [1, 2, 28]. Both in vitro [32] and in vivo [13, 26, 29, 39] studies have demonstrated that growth factors, including TGF-βs and bone morphogenetic proteins, can induce regenerative events in the dentine–pulp complex in a similar way to dentine matrix extracts naturally containing these growth factors. Exciting opportunities now exist for such studies to be extended to investigate the synergistic signalling action of several of these growth factors in tandem.

A partnership of bio-active molecules with dental materials

We are now entering a new era where our knowledge of the molecular and cellular behaviour of the dental tissues may be exploited for novel biological regenerative approaches to restorative dentistry. For this to be realised, however, such approaches need to build upon and partner with more traditional approaches using dental materials. This will not only encourage acceptance and take up by the dental profession but also allow us to adapt the best of the present technologies and extend them into the biological arena.

Effective delivery of bio-active molecules within a material to the site of regeneration is fundamental to their potential success. Direct application of pure recombinant growth factor protein in a pulp capping situation [13, 26, 29, 39] and development of a novel growth factor hydrogel capable of stimulating de novo dentinogenesis on a cut pulp surface [8] highlight the feasibility of growth-factor-mediated therapies. Nevertheless, there are some issues with such approaches since the biological half-lives of these molecules can be very short, especially in the milieu of the tissue injury site where many proteolytic enzymes will be present. The half-life of TGF-β1 is of the order of a few minutes in its free form and, unless protected from degradation, will be capable of signalling regenerative events for only a short time. Dentine, however, contains a significant store of these growth factors "fossilised" within its matrix where they are protected from degradation. Controlled gradual release of these molecules from the matrix could provide an effective means of achieving sustained signalling and also obviates the need for incorporation of expensive recombinant proteins.

Demineralising agents such as ethylenediaminetetraacetic acid [7] and lactic acid [38] are able to solubilise TGF-β1 from dentine and it has been reported that cavity etchants exhibit similar properties [34]. Etching of a cavity preparation in a human tooth allows detection of immunoreactive TGF-β1 on the treated surfaces of the cavity [41]. Together, these observations suggest that use of cavity etchants may well have effects beyond that of simply smear layer removal and facilitation of restoration adhesion but also exposure of bio-active components in the dentine matrix which could potentially signal tissue regeneration. Future studies should now be directed towards optimising the conditions of use for cavity etchants to provide effective exposure of growth factors and bio-active molecules in the dentine matrix as well as ensuring good adhesion of restorative materials to the dental tissues.

Dentistry has been a pioneer of regenerative medicine in its adoption some 60–70 years ago of calcium hydroxide as a pulp capping agent to induce tissue regeneration in the dentine–pulp complex. Despite this long history of use, the mechanisms of action of calcium hydroxide have never been satisfactorily explained. Recent evidence, however, has demonstrated that it also has limited capacity to dissolve matrix components from dentine, including TGF-β1 [11]. The solubilised matrix components are also able to modulate expression of various extracellular matrix and cytokine genes in cultured odontoblast-like cells [11] suggesting that calcium hydroxide may exert its action on tissue regeneration and dentine bridge formation through its ability to soluble bio-active signalling molecules from the dentine matrix. Recognition of this effect of calcium hydroxide opens up significant opportunities for development of other restorative materials that may be able to impact on tissue regeneration through local release of matrix-bound bio-active molecules from the dentine.

Clearly, such approaches of exploiting the partnerships between traditional materials and bio-active molecules are still in their infancy and there is much to learn about how they may most effectively be used to signal tissue regeneration in a controlled manner. However, we are now entering a new era of restorative dentistry where significant advances may be achieved through exploitation of the biology of the dental tissues.

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