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Responses of the pulp–dentin organ to dental restorative biomaterials

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Placement of a restorative material in dentin produces the possibility of pulpal injury. *In vitro* studies have shown that the constituents of dental biomaterials have toxic potentials. In the clinical setting, there may be an immediate reaction of the pulp to, for example, acid etching and to the placement of a bonding agent; however, in most cases the remaining dentin serves as protection against long-term or permanent damage to the pulp. Important factors for long-term pulpal outcome are microleakage with possible bacterial penetration and leakage products from restorative materials. Both factors are influenced by the cavity depth, i.e., the remaining thickness of sound dentin.

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Introduction

Maintenance of a healthy pulp tissue is important for tooth function and survival. Secondary and tertiary dentin production serve to protect the tooth and jaw from infections, caries, and traumatic dentin exposure, and nervous stimuli from the pulp regulate the masticatory forces and help to prevent damage during function. However, wear, trauma, and disease may impair the barrier otherwise put up by the dentin coverage, and pulpal necrosis may occur after many insults during the lifetime of the tooth. Dental caries, cavity preparation, and restorative materials may all produce harmful effects on the pulp tissue and this sequence of events may be repeated several times. Fortunately, experimental studies and clinical observations of revitalization after trauma have shown that the effects of many insults to the pulp may be reversible or repairable and do not necessarily lead to pulp necrosis.

The pulp is a connective tissue and responds as such to stimuli and insults. Inflammation is the dominant reaction, with both acute and chronic responses depending on the magnitude and duration of the insult. In addition, tissue-specific reactions such as increased dentinogenesis and increased calcification of the dentin are observed. Changes in vascular permeability occur during acute inflammation, resulting in the formation of exudates. Because of the limited space for pulp tissue to expand, the intrapulpal pressure increases, causing pain. Chronic inflammation may persist for many years, often without any discomfort to the patient.

Dentin reactions

The microscopic anatomy of native dentin is well known: dentinal tubules, with interconnecting microtubules, radiate from the odontoblasts of the peripheral pulp. They are encased in a collagen–hydroxyapatite body, the intertubular dentin. Mineralization of the peritubular structures continues with age, resulting in not only a less permeable but also a less dynamic tissue. This continual mineralization process may be hastened under a carious attack, and following the placement in prepared cavities of some dental materials, notably calcium hydroxide (1, 2). Clinically detectable changes in the biophysical characteristics of carious dentin may be observed after an indirect pulp capping with various materials (3). Such changes may also stem from changes in the pulp proper; elimination of infection

and reduced inflammation of the pulp may decrease exudation through dentin and allow mineralization processes in dentin to recur. There must be a balance between the toxic/antibacterial activities of a filling material in deep dentin applications and its ability to induce or allow mineralization of dentin to occur. While zinc oxide–eugenol is an excellent anodyne and strongly antibacterial, and thus has found clinical applications in deep dentin cavities, it does not promote continual mineralization (4). This is seen with calcium hydroxide and with some glass ionomer preparations (3, 5).

Pulpal responses

Odontoblast reactivity

Mild toxic insults to the pulp may result in increased dentinogensis, which may be regarded as a protective mechanism. Increased peritubular dentin formation narrows the dentinal tubuli through the formation of the so-called sclerotic dentin, which can be observed in X-rays in extensive cases. A common repair response to pulp injury is the formation of tertiary dentin (6). Unlike primary or secondary dentin that forms along the entire pulp–dentin border, tertiary dentin is focally produced in response to dentin injury or toxic products reaching the pulp–dentin complex. The process of tertiary dentin formation is either reactionary or reparative in origin (7). Reactionary dentin is typically produced by pre-existing odontoblasts in response to a carefully cut cavity or the presence of a restorative material. Reparative dentin, in contrast, is formed by newly differentiated odontoblastoid cells when the primary odontoblasts have been irreversibly injured. Reparative dentinogenesis is regarded to be more complex than the formation of reactionary dentin, and is seen in teeth with deep cavity preparation or pulp exposure (8) (Fig. 1). It is suggested that growth factors, especially those of the transforming growth factor-β (TGF-β) family, initiate odontoblast differentiation and stimulate dentin formation (6). TGF-β receptors are demonstrated on odontoblasts and the growth factors are found within the dentin matrix (9). Release of growth factors may occur during carious attack and other injury to the tissue, and also during subsequent cavity preparation and restoration of the tooth. Exposure both to cavity-conditioning agents and leachables from restorative materials may contribute to the release of growth factors (10).

Inflammation

Inflammation of the pulp tissue may be initiated by various toxins, necrotic cells, or stimulation of odontoblasts (11, 12). The central role of damage to the odontoblast and the release from this cell of bioactive molecules in initiating an inflammatory response in the pulp is illustrated in Figure 2. Depending on the duration and magnitude of the challenge, the inflammation takes on an acute or a chronic approach, and a chronic inflammation may be exacerbated. Mast cells observed in dental pulp are suggested to play an important role in pulpitis (13). Through the release of mediators, mast cells can exert potent chemotactic and stimulatory effects on other cell types, such as macrophages and neutrophils (14), and thus potentiate the inflammation. In clinical practice, pulpitis is often classified as reversible or irreversible. The distinction is based on the clinician's judgement of whether the pulp may recover from the insult(s) experienced (15) or if the damage is so great that the pulp is open to and defenseless against infection through, for example, caries. Irreversible pulpitis is associated with episodes of pain and clinical or radiographic evidence of pulpal exposure or near exposure. Elevated levels of tumor necrosis factor-α (TNF-α) are found in irreversible pulpitis (16). TNF-α is known to increase the toxicity of leukocytes, stimulate the synthesis of acute phase inflammation proteins, and induce the expression of other proinflammatory cytokines (17). Together with other cytokines, pulp-derived TNF-α stimulates human pulp cells to synthesize and secrete proteolytic enzymes that destroy the extracellular matrix (18–21). Figure 3 shows a histological slide illustrating many of the pulp responses to noxious stimulation of dentin.

Cavity depth (remaining dentin thickness)

The significance of the remaining dentin thickness beneath the cavity preparation to prevent pulp injury has long been recognized, although the quantitative relationships between dentin thickness and the risk of pulpal injury remain uncertain (22). It seems that odontoblast survival is most sensitive to the remaining

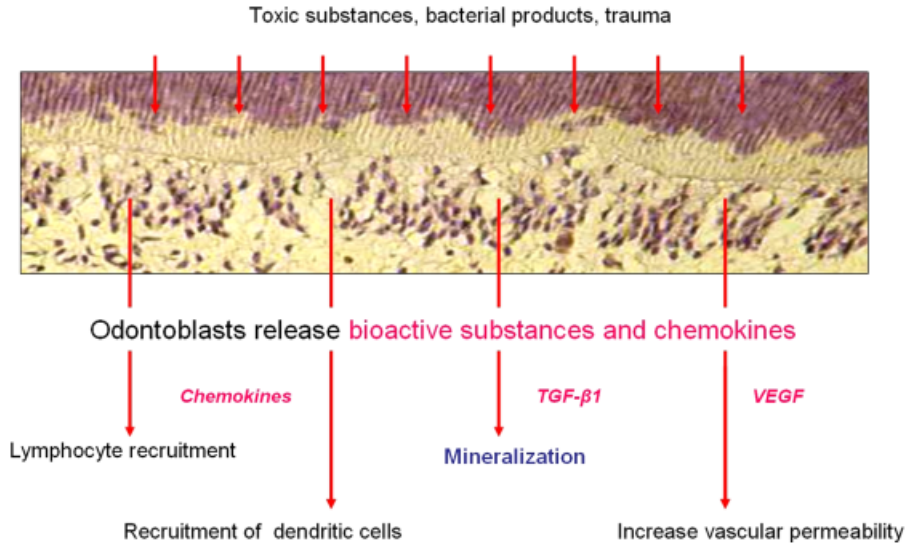

Fig. 1. Odontoblast response to pathological stimulation. TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor. Adapted from Smith et al. (7).

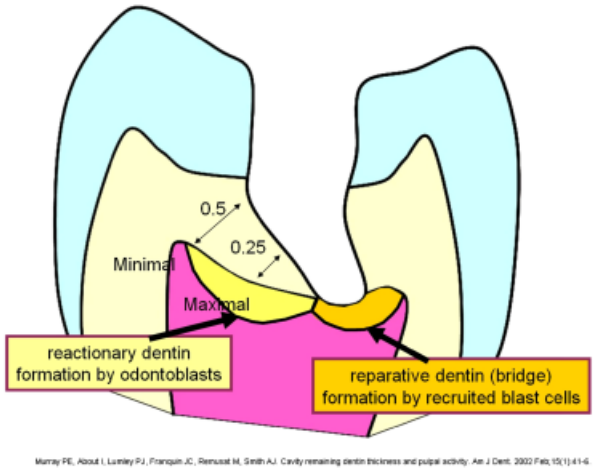

Fig. 2. Dynamics of hard tissue formation by the pulp in response to external stimuli at various depths of dentin exposure. Reactionary dentin formation increases with decreasing residual dentin thickness down to some 0.1 mm; at closer exposures, necrosis of odontoblasts results in reduced reactionary dentin formation. Reparative dentin formation then remains as the only, albeit ineffective, means of producing a bridge, sealing off the irritant from the remaining pulp. Based on Mjör (1).

dentin thickness. A dentin thickness of 1 mm has been suggested to protect the pulp from the harmful constituents of dental materials (23). This has later been modified, indicating that preparations carefully cut down to 0.5 mm from the pulp tissue had only a limited effect on the underlying odontoblast survival rate, assuming the absence of bacteria (24).

Pulpal effects of restorative materials

Adhesive resins

Adhesive resins dominate as dental filling materials. The pulpal effects of these materials can be divided into two scenarios: the immediate effects of cavity treatment and acid etching, and the prolonged effects of leachables from the restorative materials. Two main concepts for etching the dentin and enamel are currently in use: *etch-and-rinse* and *self-etch* (25). In the etch-and-rinse technique, strong phosphoric acid is applied to the cut cavity surface and rinsed off with water spray before application of a primer. Self-etching adhesives contain acidic monomers that are combined with the monomers of the primer. The acidic component is not rinsed off but is neutralized by dentinal components. Common constituents of bonding systems are 2-hydroxyethylmethacrylate (2-HEMA), triethyleneglycol dimethacrylate, and urethane dimethacrylate (26); these are also found in resin-based restorative materials together with bisphenol-A diglycidylether methacrylate and ethoxylated bisphenol-A dimethacrylate. It is recognized that many of these substances may have significant toxic effects, some of which may be important in their clinical performance (27).

Immediate effects

Experimental studies on rats using a vital microscopic technique have shown that acid etch and bonding

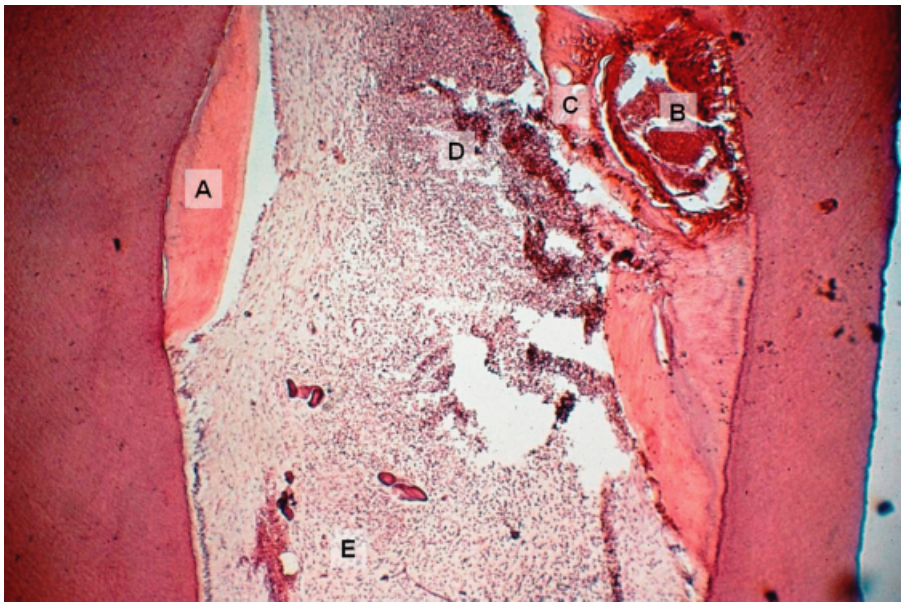

Fig. 3. Histologic slide showing many of the elements of pulp reactions after dentin exposure to toxic and infectious agents. (A) Reactionary dentin; (B) odontoblast and pulp tissue necrosis; (C) reparative dentin (bridge) formation by recruited blast cells; (D) intense inflammation (mixed acute and chronic) subjacent to the incomplete bridge, yet relatively localized in view of the near-normal pulp tissue more apically (E). From the collection of S. Seltzer.

materials applied to the dentin caused vasodilatation in pulpal tissue, and that the effect was more pronounced after acid etch and bonding than after bonding alone (28). However, vasoconstriction was observed when a self-etching product was tested with the same methodology (29). Changes in pulp microcirculation may induce nerve signalling responses. In clinical studies, the frequency of post-operative sensitivity has been used to evaluate the pulpal effect of various bonding systems. Unemori et al. (30) compared an etch-and-rinse bonding product and a self-etch bonding product and found that in deep and medium-deep cavities the incidence of post-operative sensitivity was much lower in teeth where the self-etch product was used. It is suggested that self-etching bonding products dissolve the dentin partially, such that a substantial number of hydroxyapatite crystals remain within the hybrid layer (31). Specific carboxyl or phosphate groups of functional monomers can then chemically interact with this residual hydroxyapatite (31), creating a protective surface. In addition, self-etching bonding products do not penetrate as deeply into the dentin surface as the bonding which occurs after the etch-and-rinse technique (32). Both of the above aspects would indicate less post-operative sensitivity. In another study, no post-operative sensitivity was observed for restorations placed with the different etching techniques (33), and it has been suggested that post-operative sensitivity may be more related to stress caused by polymerization contraction rather than the type of adhesive used (34).

Placement of dental adhesives on intentionally exposed human pulp has resulted in inflammation of variable severity (35–39). A review on the current status of pulp capping with dentin adhesives concluded that such treatment was contraindicated (40). Inflammation was also observed in the pulp after placement of dental adhesives in deep cavities where only a thin dentin wall separated the pulp and the adhesives (35, 41). Eluates from dental adhesives are toxic to human primary pulp cells *in vitro* (42), and a number of constituents included in the adhesives have been found to be cytotoxic in other cell systems (8, 43–47). Dental adhesives cause coagulation of the blood vessels of the chorio-allantoic membrane of hen eggs, suggesting the ability to injure mucous membranes (26, 48).

It can be concluded that even if the etchant and the bonding materials have the potential for biological effects, the pulp tissue is protected by the dentin. Immediate pulpal effects of etching and bonding procedures are of concern only in cavities with a thin dentin barrier.

Long-term effects

Microleakage and bacteria, in addition to cavity depth, are believed to play important roles for pulpal involvement,

maybe more so than the restorative material (49–51). Cavity depth is an important factor for the expression of any toxic influences. However, in the presence of bacteria in the cavity-restoration margins, the pulpal response is related neither to the type of the restorative material nor to the cavity depth (50, 51). Consequently, if optimal conditions for the preservation of pulpal health are to be ensured, dental restorations should provide a resistant seal along the cavity margins (49).

Even then, the restorative material cannot be completely acquitted of causing pulpal problems for the patient. Resin-based materials are composed of filler-reinforced monomers that polymerize, and complete polymerization is never achieved. Normally, 25–50% of the monomer double bonds remain unreacted in the polymer (52, 53). It has been shown that monomers leach out of resin-based material (54), and they may pose a risk to the pulp of the tooth if the leachables pass through the dentin (55). Few studies have addressed the long-term pulpal outcome of dental restorative materials. In one study with observation times of up to 36 months, clinically verifiable pulp damage was more common in teeth with deep cavities compared to moderately deep or shallow cavities and in teeth restored with composite resin compared to amalgam (56). The application of a calcium hydroxide lining or resin bonding system was not a critical determinant of pulp outcome as of 36 months post-restoration (56).

From these studies it was again suggested that the long-term marginal integrity of the restoration seems to be of fundamental importance for the maintenance of a healthy pulp (56). Amalgam restorations are free from polymerization shrinkage which may compromise the integrity of the tooth/restorative interface. Composite resins, on the other hand, are technique sensitive and require incremental build-up to prevent potentially excessive dimensional change during application. While it is possible using careful technique to develop tight interfaces with dental tissues in the short term, this may not reflect the material's long-term clinical performance. Hydrolytic degradation of the bonding interface (57) and thermal and mechanical stress may weaken and disrupt the bond, leading to microleakage.

Conventional glass ionomer cement

When glass ionomer cements were introduced in the market, their pulpal responses were described as bland, moderate, and less irritating than responses to zinc phosphate cement and resin composites (58). Since then, the biocompatibility aspects of glass ionomer cements have been intensively studied. Glass ionomer fillings were reported to be non-toxic to pulp tissue if bacterial penetration was avoided (59). Also, the cytotoxicity of fully set glass ionomers was shown to be minimal (60). However, glass ionomer cements appeared to be pulp irritants when used as luting agents (58), but there are no such reports in the more recent literature (60). It is therefore unclear if the previous recommendation to protect the pulp tissue using calcium hydroxide paste on areas of crown preparations that appear to be close to the pulp (< 1.0 mm) before the luting procedure is carried out is still valid.

Resin-modified glass ionomer

Stronger and less brittle hybrid materials have been produced by the addition of hydrophilic monomers such as HEMA, capable of free radical polymerization (e.g. via light-curing) on conventional glass ionomer cements. These resin-modified preparations proved to be cytotoxic mainly due to the release of high amounts of HEMA (60, 61) and were also observed to be mutagenic. However, the mutagenicity data are sparse and difficult to interpret (60). Pulp response studies of resin-modified glass ionomers have shown conflicting results. In one study, almost no effects were observed in the pulp tissue below resin-modified glass ionomer fillings, and a transient inflammatory response was followed by dentin bridge formation in pulps directly exposed to the material (62). The overall conclusion was that resin-modified glass ionomers showed acceptable biological behavior toward both exposed and non-exposed pulps. In another study, a resin-modified glass ionomer was compared with calcium hydroxide as the pulp capping material (63). The resin-modified glass ionomer caused a moderate to intense, persistent inflammatory response in the pulp, together with the formation of a large necrotic zone (63).

Temporary filling materials

Among these, zinc oxide–eugenol-based formulas dominate. They have a long history as seal-tight and,

when reinforced, durable restorations which, at the same time, provide a pharmacological effect by their release of eugenol. While it may be seen as contradictory that eugenol, a highly cytotoxic substance (64), should be considered bland to the dental pulp, this is again a reflection of the importance of the remaining dentin thickness. It has been shown (65) that eugenol diffuses very poorly across dentin. On the cavity side, the toxicity of eugenol is advantageous as it takes on antibacterial properties, whereas the much lower concentration reaching across dentin to the pulp may serve as a mild analgesic and anodyne to that tissue (66, 67).

Cavity bases and materials for direct pulp capping

Cavity base materials are designed for the explicit purpose of protecting the pulp from damage by the influences mentioned above: material components, antigens, and microbes. They serve as reinforcements of the residual dentin barrier. Because structural integrity is not a primary issue with these materials, one may avoid the many components associated with adverse effects of composites and other restorative materials. The potential ability of these materials to induce tertiary dentin formation was believed to be important for their function, and calcium hydroxide-based materials gained popularity because of this. Current research suggests that this ability is mediated through the release of growth factors and other bioactive molecules from the dentin by $Ca(OH)_2$ (68). However, there may be an even stronger focus on the antibacterial properties of cavity bases. The formation of tertiary dentin may be seen as a default response by the pulp to dentin injury, a response that, in the absence of bacteria at the dentin surface, may proceed uninhibited. Whereas many materials applied to dentin, including some with antibacterial substances such as eugenol, are toxic to pulp cells, calcium hydroxide is apparently sufficiently antibacterial on the dentin surface while at the same time bland to pulp cells or stimulates them to dentin production. Various biologically active substances are being tested for their possible stimulatory effect on dentin production in deep cavities (69). Indeed, a number of substances have been shown to have potential as dentin-stimulating agents (70).

Calcium hydroxide is also a reference as a pulp capping material, i.e., as a base on pulpal tissue exposed by trauma. Here, the induction of tertiary dentin occurs through activation of blast cells in the pulp. These are recruited as new hard tissue-forming cells replacing the odontoblasts that were destroyed by the exposure. In this clinical application, calcium hydroxide produces a zone of superficial necrosis which serves as a scaffold for an underlying hard tissue repair, forming a 'dentin bridge.' The bridge is seldom continuous and complete, and the barrier effect is compromised correspondingly. Several bioactive substances have also been experimentally tested for intended use as pulp capping and reparative dentin-forming materials, and some products have indeed shown some potential (70). Moreover, a more predictable effect on the pulp may be obtained with mineral trioxide aggregate (MTA), a material for which we are finding ever-new applications in operative dentistry and endodontics. Animal experiments have shown that this material seems to be very suitable for pulp capping and probably also pulpotomy procedures (71). MTA uses Portland cement as the parent compound and its biocompatible nature is related to the formation of hydroxyapatite when exposed to physiological solutions (72). MTA has also shown excellent potential as pulp capping and pulpotomy materials in clinical studies. In preliminary studies, MTA has demonstrated favorable use as apical and furcation restorative materials. However, these findings need to be evaluated with longer time perspectives; studies in these areas with long-term follow-up are currently limited (72).

Concluding remarks

It is not documented that leachables from dental biomaterials pose a great risk to the pulp except in cases where the remaining dentin is very thin. For long-term preservation of a healthy pulp in a restored tooth, the use of minimally invasive techniques and dental biomaterials that maintain a good seal against leakage are advised. Materials which combine antibacterial activity with little toxicity to pulp cells are suitable for the repair of superficial pulp damage, and the controlled stimulation of pulp cells to produce hard tissue appears to be a realistic treatment for the near future.

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