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Reactionary dentinogenesis

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ABSTRACT Reactionary dentinogenesis is the secretion of a tertiary dentine matrix by surviving odontoblast cells in response to an appropriate stimulus. Whilst this stimulus may be exogenous in nature, it may also be from endogenous tissue components released from the matrix during pathological processes. Implantation of isolated dentine extracellular matrix components in unexposed cavities of ferret teeth led to stimulation of underlying odontoblasts and a response of reactionary dentinogenesis. Affinity chromatography of the active components prior to implantation and assay for growth factors indicated that this material contained significant amounts of TGF-ß$_1$, a growth factor previously shown to influence odontoblast differentiation and secretory behavior. Reactionary dentinogenesis during dental caries probably results from solubilization of growth factors, TGF-ß in particular, from the dentine matrix which then are responsible for initiating the stimulatory effect on the odontoblasts. Compositional differences in tertiary dentine matrices beneath carious lesions in human teeth have also been shown indicating modulation of odontoblast secretion during reactionary and reparative dentinogenesis.

KEY WORDS: *dentinogenesis, extracellular matrix, odontoblasts, growth factors, dental caries*

Introduction

Whilst the ability of odontoblasts to react to appropriate stimuli (e.g., disease, chemicals or trauma) and secrete a tertiary dentine matrix at specific foci has long been recognized, there has often been little attention paid to the exact nature of the underlying biological processes giving rise to such responses. Recognition of reactionary and reparative variants of tertiary dentinogenesis as being distinct processes has allowed consideration of the molecular mechanisms underlying such pathological processes and comparison with the primary dentinogenesis of physiological tooth development.

This paper initially considers the chronology of dentinogenesis as a foundation to investigating these molecular mechanisms and reflection as to whether reactionary dentinogenesis represents a distinct pathological process or if it constitutes one end of the spectrum of physiological processes. Elucidation of these processes will not only allow a better understanding of the behavior of odontoblasts, but also provide an impetus for exploitation in the development of new biomaterials for improved clinical management of dental disease with a sound scientific basis.

Chronological considerations of dentinogenesis

The odontoblast is recognized as a post-mitotic cell, which differentiates at the late bell stage of tooth development. During dentinogenesis, the prime function of this cell is synthesis and secretion of the extracellular matrix (ECM) components of dentine and subsequent mineralization of the matrix. Despite this secretory role, the morphological appearance of the odontoblast can vary widely throughout its life cycle (Takuwa and Nagai, 1971; Fox and Heeley, 1980; Couve, 1986; Romagnoli *et al.*, 1990). Under physiological conditions, this morphological appearance can be related to the secretory activity of the cell, but pathological changes within the tissue can lead to morphological changes in the odontoblast. These changes may suggest far less of a relationship between morphology and secretory behavior in that odontoblasts often lose their columnar appearance under these conditions, but may secrete a tertiary dentine matrix (albeit often dysplastic) at quite high rates (Baume, 1980).

During tooth development, the dentine secreted up until the completion of root formation may be defined as primary dentine and will comprise the main bulk of the circumpulpal dentine matrix in the human tooth. This forms at a rate of approximately 4 microns per day (Schour and Poncher, 1937; Massler and Schour, 1946; Kawasaki *et al.*, 1980), although the rate of dentine formation is slower in the root than the crown of the tooth. Physiological secondary dentine is laid down after completion of root formation and occurs at a much slower rate throughout life (Baume, 1980). The relative positions of primary and secondary dentines are

Abbreviations used in this paper: TGF-ß, transforming growth factor ß; BMP, bone morphogenetic protein; IGF, insulin growth factor; EDTA, ethylenediaminetetraacetic acid; ECM, extracellular matrix.

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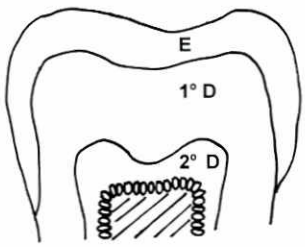

Fig. 1. Schematic illustration of the positions of primary dentine (1°D) and physiological secondary dentine (2°D) relative to enamel (E) in the human tooth.

indicated in Fig. 1. Deposition of secondary dentine within the pulp chamber can often be seen to occur in an asymmetric manner, e.g., in molar teeth, greater deposition is often seen on the roof and floor of the pulp chamber.

The appearance of the matrix of primary dentine indicates a regular, tubular structure and that of secondary dentine is not dissimilar, although it has been suggested that there is slightly less tubular structure in the latter (Mjör, 1983). The matrix of dentine, however, comprises both intertubular and peritubular (intratubular) dentine, the compositions of which show some distinct differences (Baume, 1980). Peritubular dentine contains few collagen fibrils, whilst intertubular dentine has a rich meshwork of collagen fibrils within it (Jones and Boyde, 1984). Dentin phosphoproteins are absent from predentine (Jontell and Linde, 1983; MacDougall *et al.*, 1985; Nakamura *et al.*, 1985; Gorter de Vries *et al.*, 1986; Takagi and Sasaki, 1986; Rahima *et al.*, 1988) and appear to be more concentrated within peritubular dentine (Aulak *et al.*, 1991). Such observations have led to the suggestion (Linde, 1989) that two sites of secretion exist in the odontoblast – one at the level of the cell body to produce predentine and a second at the mineralization front giving rise to peritubular dentine. This hypothesis helps to explain many of the compositional differences within the matrix of dentine in different regions. It also indicates the complexity of the secretory process in dentinogenesis. The autoradiographic and ultrastructural evidence for this hypothesis has recently been reviewed (Linde and Goldberg, 1993) and whilst it lends support to the concept of two levels of secretion, a number of questions remain to be addressed. For instance, further information is needed regarding whether secretion is mediated by vesicles at both levels and, if so, how are the vesicles differentially targeted at the two levels? Little understanding exists of the regulation of these processes, which could occur at the transcriptional, translational or post-translational levels. It is also important to distinguish between secretory vesicles and endocytotic material in studying odontoblast secretion.

Secretion of matrix at the level of predentine can be seen as an increasing overall thickness of primary or physiological secondary circumpulpal dentine depending on the chronological stage of the tooth. Secretion of matrix at the mineralization front (and possibly from other points along the odontoblast process) will give rise to a progressive increase in the thickness of the peritubular dentine layer. It has not been possible to distinguish between the peritubular dentine matrix secreted at different points in the chronological development of the tooth, e.g., that secreted during primary or secondary dentinogenesis. Dentinal tubules show a tapered structure due to deposition of peritubular dentine and their gradual occlusion is a feature of the aging process giving rise to sclerotic dentine (Ten Cate, 1994).

Tertiary dentine is generally recognized as representing a dentine matrix laid down at specific loci of the pulp-dentine interface in response to environmental stimuli. Such stimuli, if of relatively mild degree, will stimulate an increased rate of matrix secretion by the existing odontoblasts exposed to influence. Stronger stimuli, however, will lead to death of the odontoblasts and if conditions in the dentine-pulp complex are favorable, a new generation of odontoblast-like cells secreting a reparative dentine matrix will differentiate. In an attempt to recognize the different sequences of events in these two situations, we have proposed the following definitions (Lesot *et al.*, 1993; Smith *et al.*, 1994).

Reactionary dentine: a tertiary dentine matrix secreted by surviving post-mitotic odontoblast cells in response to an appropriate stimulus.

Reparative dentine: a tertiary dentine matrix secreted by a new generation of odontoblast-like cells in response to an appropriate stimulus, after the death of the original post-mitotic odontoblasts responsible for primary and physiological secondary dentine secretion.

These tertiary dentines are schematically depicted in Fig. 2. The fundamental point in these proposals is that the tertiary dentine secreted (reparative or reactionary dentine) arises from two different populations of cells, i.e., the original post-mitotic odontoblasts responsible for primary dentinogenesis and a new generation of odontoblast-like cells, which have differentiated from pulpal precursor cells as a reparative mechanism for tissue repair.

The structure of tertiary dentines can be very variable with a spectrum of appearance ranging from a regular tubular matrix which is virtually indistinguishable from primary dentine to a very dystrophic, atubular matrix possibly containing cells trapped within it. Furthermore, not only is tertiary dentine laid down at the pulp-dentine interface, thereby increasing the overall thickness of circumpulpal dentine, it can also be observed as an increased rate of peritubular dentine deposition. It is important, however, to distinguish this increased peritubular dentine deposition within tubules from intra-tubular calcifications (Frank *et al.*, 1964; Mjör and Furseth, 1968; Selvig, 1968). The latter are rather poorly

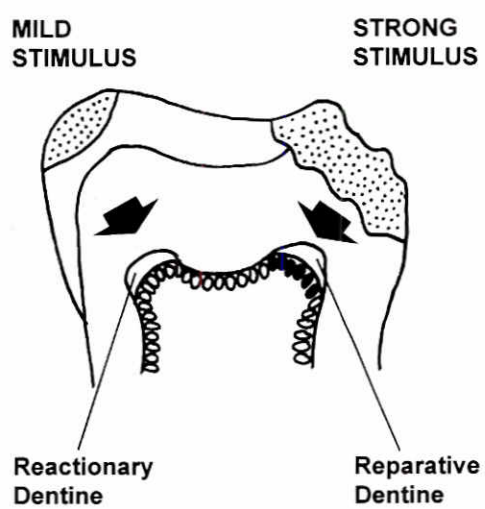

Fig. 2. Schematic illustration of reactionary dentine deposition in response to mild (e.g., early caries, mild abrasion/erosion) and strong (e.g., severe caries) stimuli respectively. *Note that reactionary dentine is secreted by surviving post-mitotic odontoblast cells (white), whilst reparative dentine is secreted by a new generation of odontoblast-like cells (black) after death of the original post-mitotic odontoblasts responsible for primary dentine secretion.*

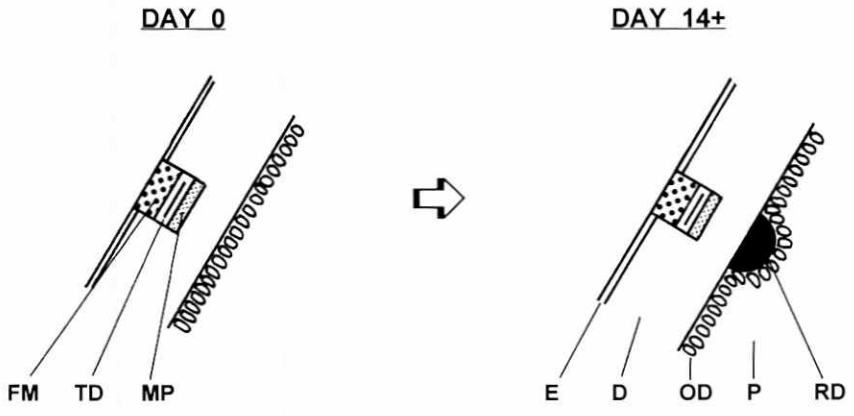

Fig. 3. Schematic illustration of implantation of an extracellular matrix fraction isolated from dentine (MP) into unexposed cavities in ferret teeth. *After 14+ days, significant deposition of reactionary dentine (RD) by the odontoblasts (OD) immediately underlying the cavity and in direct contact through their dentinal tubules was observed. (FM, filling material [Kalzinol]; TD, inert teflon disc; E, enamel; D, dentine; P, pulp).*

understood phenomena, which appear to be distinct from the normal dentinogenic events (Baume, 1980). These intra-tubular calcifications have been reported in the translucent zone of dentine caries (Frank *et al.*, 1964) and in calcium hydroxide covered dentine (Mjör and Furseth, 1968) and appear to represent a physicochemical precipitation of hydroxyapatite or whitlockite crystals rather than a vital process. Such a view is supported by *in vitro* dissolution experiments in dentine by weak acids (Selvig, 1968) in which reprecipitation of the dissolved mineral salts was seen as intratubular crystalline deposits of whitlockite and hydroxyapatite. Occlusion of dentinal tubules by peritubular dentine deposition beneath carious lesions leading to dead tract formation is a not uncommon observation (Ten Cate, 1994). Thus, the peritubular dentine matrix may comprise secretions arising from primary, secondary or tertiary dentinogenesis. Also, the order of secretion of these accumulated matrices may vary, e.g., tertiary deposition at peritubular sites may feasibly be interposed between primary and secondary dentine secretions, although it might be expected that a primary, secondary, tertiary sequence would be more common. Thus, any consideration of tertiary dentinogenesis within peritubular areas is complicated by the phenomenon of physiological dentinogenesis, whether it be primary or secondary, the latter of which is a normal feature of the ageing process. It is only really at the pulp-dentine interface that reliable distinction can be made between tertiary and other dentines.

Tertiary dentinogenesis: reactionary is distinct from reparative dentinogenesis

To define a tertiary dentine as reactionary or reparative, it is necessary to have chronological information on the previous events within the pulp-dentine complex (Lesot *et al.*, 1993). In this way, it is possible to determine whether the tertiary dentine matrix has been secreted by the post-mitotic odontoblasts arising during normal tooth development or by a new population of odontoblast-like cells that have differentiated after death of the original odontoblasts as a result of disease or trauma. Whilst the presence of tertiary dentine, secreted in response to a variety of stimuli, has been widely described in human teeth it is very difficult to distinguish whether it is reactionary or reparative in nature. In the absence of the previous history of these teeth, in terms of cell behavior in the dentine-pulp complex, it can only be concluded that a tertiary dentine of unknown origin has been secreted. Because of the episodic nature of dental caries, it is likely that the tertiary dentine beneath a carious lesion may often comprise both reactionary and reparative dentines superimposed upon one another. Thus, at the *in vivo* level, it is difficult to investigate the process of reactionary dentinogenesis in human teeth.

Animal models offer certain advantages for the examination of reactionary dentinogenesis *in vivo* where time course studies on the processes occurring can be undertaken. Implantation of a lyophilized extracellular matrix fraction isolated from dentine into unexposed cavities in ferret teeth gave rise to stimulation of the odontoblasts' synthetic and secretory activity (Smith *et al.*, 1994b). This is schematically illustrated in Fig. 3. With implantation periods as short as 14 days, there was significant deposition of reactionary dentine at the pulp-dentine interface by the odontoblasts immediately underlying the cavity and in direct contact through their dentinal tubules. In contrast, control cavities lacking the dentine matrix components showed no evidence of reactionary dentine deposition. Identification of the response as one of reactionary dentinogenesis by stimulation of existing odontoblasts was confirmed by examination of teeth at early periods of implantation (2 and 5 days), which indicated that odontoblast death had not occurred as a result of the operative procedures. The absence of odontoblast death in these teeth is a reflection of the carefully controlled method of cavity preparation since such preservation may be harder to achieve during cavity preparation under normal clinical conditions. In a recent review of the influence of various dental materials on odontoblast activity, it was concluded that the cavity preparation procedures had a greater influence on the odontoblast activity than the materials themselves (Cox *et al.*, 1992). This is borne out by the observations that, even using the same cavity preparation procedures as above, the response of reactionary dentinogenesis beneath cavities filled with various dental materials was not nearly so strong as that seen after implantation of the dentine ECM components (Plant *et al.*, 1991; Tobias *et al.*, 1991). Thus, under carefully controlled conditions it is possible to prepare a cavity in a tooth, maintain odontoblast integrity and subsequently stimulate these odontoblasts by implantation of isolated dentine ECM components to secrete a reactionary dentine matrix.

The absence of odontoblast death in the above study on implantation of dentine ECM components implies that reactionary dentinogenesis can result from interaction between existing odontoblasts and appropriate molecular stimuli leading to an increase in the synthetic and secretory activity of the odontoblast. This contrasts markedly with reparative dentinogenesis, where

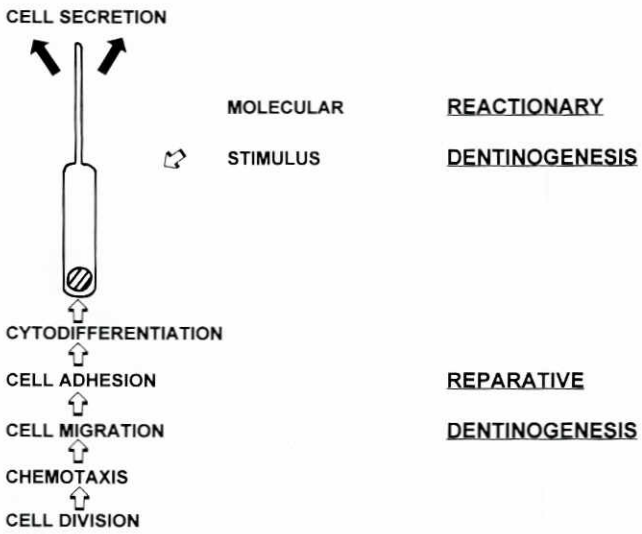

Fig. 4. Schematic illustration of the biological events underlying reactionary and reparative dentinogenesis. *Reactionary dentinogenesis requires only interaction between the molecular stimulus and the odontoblast for stimulation of matrix secretion, whilst reparative dentinogenesis requires a cascade of events terminating in cytodifferentiation of a new generation of odontoblast-like cells before secretion of matrix can occur.*

odontoblast death occurs and appropriate pulpal precursor cells are induced to differentiate into a new population of odontoblast-like cells (Smith *et al.*, 1990). It is possible that similar molecules are involved in the initiation of reparative and reactionary dentinogenesis since the same fraction of isolated dentine ECM components can give rise to both of these processes. However, the biological events underlying these processes are different (Fig. 4). In reparative dentinogenesis, a cascade of events involving cell division, chemotaxis, cell migration, cell adhesion and cytodifferentiation must occur before secretion of the matrix can take place. Reactionary dentinogenesis, however, requires only interaction between the molecular stimulus and the odontoblast for stimulation of secretion of the matrix.

After implantation of isolated dentine ECM components (Smith *et al.*, 1994), the deposition of reactionary dentine at the pulp-dentine interface increased in a non-linear manner with time of implantation. This may have been a result of the active component(s) becoming limiting due to the dose-response behavior of the odontoblasts and/or degradation of the active component(s). The response of reactionary dentinogenesis decreased with increasing thickness of residual dentine beneath the cavities, probably as a result of the increased distance of diffusion from the cavity to the odontoblasts down the dentinal tubules. Thus, the distance of diffusion may limit odontoblast stimulation either due to degradation or dose-response. This response limiting factor of the distance of diffusion may suggest that the interaction of the active component(s) occurs on the odontoblast cell body rather than on the odontoblast process where distance of diffusion would be less critical.

Whilst the same fraction of isolated dentine ECM components can initiate both reparative (Smith *et al.*, 1990) and reactionary (Smith *et al.*, 1994) dentinogenesis, it is probable that a synergistic association of components is required to initiate the cascade of events in reparative dentinogenesis (see paper by Tziafas in this issue). Reactionary dentinogenesis, however, appears to be a less complex biological process and may have much simpler requirements in terms of the initiating molecule(s). To investigate the nature of the molecules involved in the initiation of reactionary dentinogenesis, we have further purified the EDTA-soluble fraction of dentine matrix components by affinity chromatography on heparin-agarose. The bound material, eluting with 0.15 M-NaCl (similar affinity as certain members of the transforming growth factor ß family) was assessed for its ability to initiate reactionary dentinogenesis and assayed for the presence of TGF-ß. After 14 days implantation in unexposed cavities prepared in ferret canine teeth (Fig. 5a), this material (containing approximately 115 pg TGF-$ß_1$ per implantation site) stimulated secretion of a tubular reactionary dentine matrix at the pulp-dentine interface immediately beneath the cavities (Fig. 5b). The interface between the reactionary dentine and odontoblasts was irregular and overall, the reactionary dentine often had the appearance of finger-like projections (Fig. 5c) suggesting that closely adjacent odontoblasts had responded to different extents to the stimulation from the implanted material. The overall appearance of cellular inclusions within the reactionary dentine matrix was largely the result of the arrangement of the finger-like projections rather than being true cellular inclusions. Deposition of reactionary dentine in the experimental cavities was only seen in those areas where the dentinal tubules were in communication with the cavity indicating the role of diffusion of the molecules through the dentinal tubules (Fig. 5d). Control cavities (Fig. 5e) lacking the implanted material showed no evidence of reactionary dentinogenesis.

Use of animal models appears to be the most fruitful way to examine reactionary dentinogenesis *in vivo*, but recent studies indicate that *in vitro* models could be valuable in this area. Slices of rodent incisor teeth, in which the dentine-pulp complex tissue architecture is maintained, have been successfully cultured for periods up to 14 days (Sloan *et al.*, 1994) in a semi-solid agar based medium and this approach also appears feasible for culture of human tooth slices. Human tooth slices have also been cultured in liquid media for similar periods of time (Magloire *et al.*, personal communication). These culture models have significant potential for investigating reactionary dentinogenesis, where odontoblast behavior can be examined independently of the normal inflammatory processes which can occur *in vivo*.

Variations in the odontoblast phenotype

The morphological appearance of the odontoblast and the tubular matrix it secretes have been some of the most characteristic features of this cell. Whilst certain differences in the composition of dentine and the closely related hard-tissue, bone, can be observed, it is not clear whether these are characteristic enough on an individual molecular basis to be used as phenotypic markers. Even morphological appearance is fraught with problems since the odontoblast cell changes in appearance through its life-cycle (Takuwa and Nagai, 1971; Fox and Heeley, 1980; Couve, 1986; Romagnoli *et al.*, 1990) and the regularity of the tubular structure varies from primary to tertiary dentines. *In situ* hybridization has allowed comparison of the expression of various transcripts in normal odontoblasts and odontoblast-like cells induced by various growth factors (Bègue-Kirn *et al.*, 1994). These studies have shown that odontoblasts and odontoblast-like cells, which are

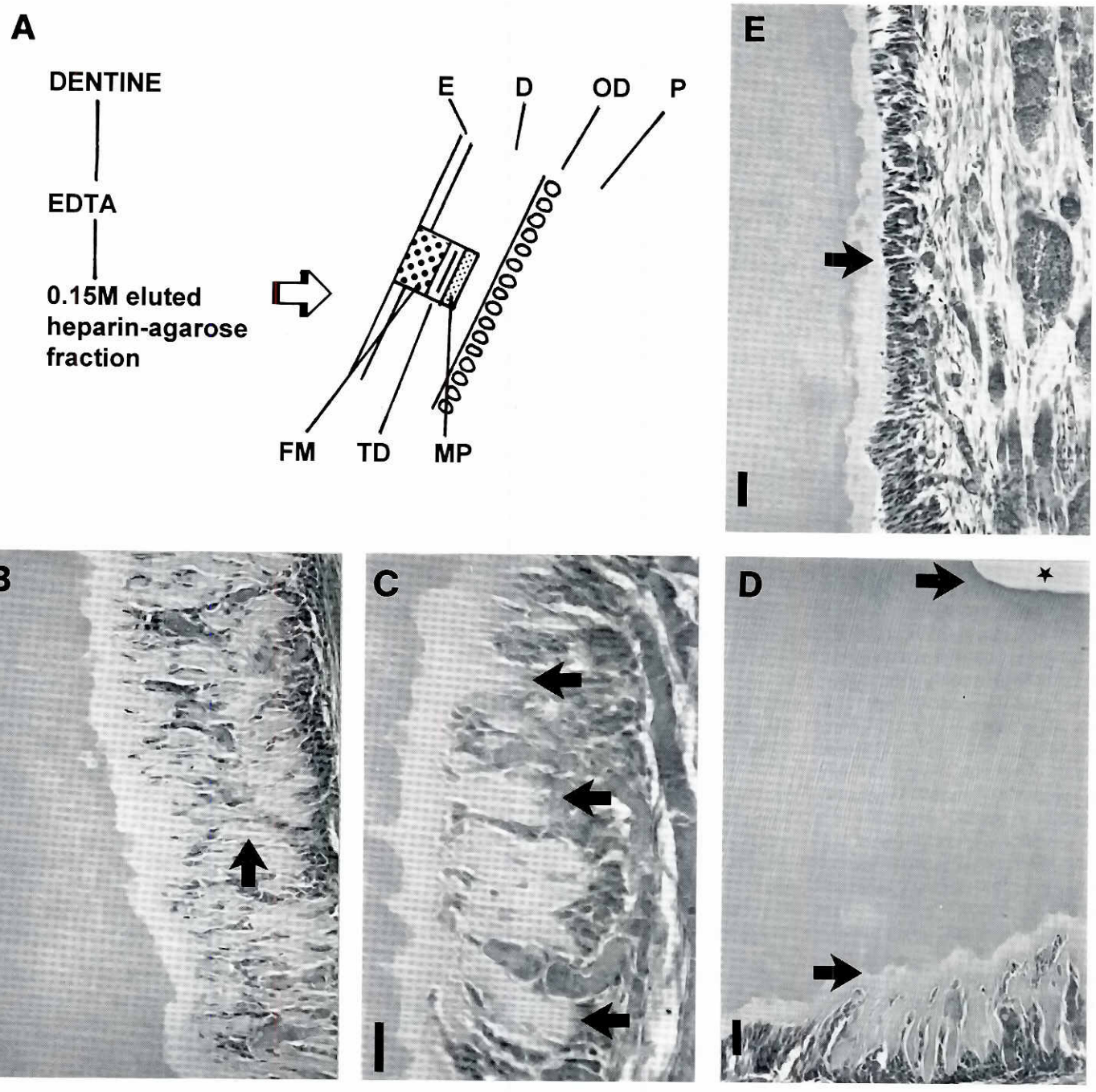

Fig. 5. Implantation of dentine matrix fraction (MP), purified by heparin-agarose affinity chromatography, in unexposed cavities in ferret canine teeth. (A) *Schematic illustration of implantation (FM, filling material [Kalzinol]; TD, inert teflon disc; E, enamel; D, dentine; OD, odontoblasts; P, pulp).* **(B-D)** *Pulp-dentine interface beneath experimental cavities 14 days after implantation of the dentine matrix fraction.* **(B)** *Significant deposition of tubular reactionary dentine matrix (arrow) immediately beneath the cavity, x200.* **(C)** *Irregular interface between the reactionary dentine and odontoblasts with the appearance of finger-like projections (arrows), x320.* **(D)** *Secretion of reactionary dentine was limited to only those areas where the dentinal tubules were in communication with the cavity floor (arrows indicate outer limits of communication at cavity and pulp-dentine interface), x200.* **(E)** *Pulp-dentine interface beneath a control cavity lacking the implanted matrix fraction and showing no evidence of reactionary dentinogenesis, x200.* **(B-E)** *Black bars at bottom left of Figs. represents 10 microns).*

characteristic of primary and tertiary dentinogenesis, have the potential for elaboration of similar matrix components. Normal odontoblasts and odontoblast-like cells induced by immobilized TGF-ß1 or BMP-2 expressed transcripts encoding for TGF-ß1 and 3, BMP-2 and -4, bone sialoprotein and osteonectin, whereas either ubiquitous expression or no expression could be detected for TGF-ß2, IGF-1 or fibronectin mRNAs. Odontoblast-like cells obtained in the presence of immobilized IGF-1, however, did not

express TGF-ß1 transcripts and only weakly expressed TGF-ß3 transcripts. Thus, use of a panel of markers may be necessary to characterize the phenotype of odontoblasts and odontoblast-like cells.

The changes in activity of the odontoblast throughout its life and the stimulation of its synthetic and secretory activity in response to appropriate stimuli resulting in reactionary dentine deposition indicate that the odontoblast is a very versatile cell. Morphological differences in the tubular structure of the matrix secreted by these cells in primary, secondary and tertiary dentines could indicate molecular changes in these matrices. An increase in the secretion of extracellular matrix has been demonstrated in tertiary dentine beneath carious lesions in human teeth (Lesot *et al.*, 1993; Perry and Smith, unpublished observations) and immunohistochemical approaches have shown re-expression of collagen type III, fibronectin and the membrane 165 kDa fibronectin binding protein in this tissue (Magloire *et al.*, 1988, 1992), although these three proteins have a transitory expression during the differentiation of primary odontoblasts (Lesot *et al.*, 1990; Lukinmaa *et al.*, 1991). Differences in the expression of phosphoproteins in primary, secondary and tertiary dentines have also been reported (Takagi and Sasaki, 1986; Aulak *et al.*, 1991). It seems probable that these various differences in matrix composition reflect modulation in odontoblast activity.

To investigate this further, the EDTA-soluble collagen, noncollagenous protein and glycosaminoglycan levels have been analyzed in reactionary/reparative dentines beneath carious lesions in human teeth (Perry and Smith, unpublished observations). The tertiary dentine specimens contained up to four times greater levels of these components compared with primary dentine. However, these levels were not uniformly raised for the three classes of matrix components suggesting that there had been differential stimulation of the synthetic/secretory pathways for these components. Thus, it is clear that modulation of odontoblast activity can occur and that variation in expression of the different ECM components can result. Attempts to relate these compositional changes to the degree of regularity of the tissue tubular structure indicated little relationship between these parameters. It is now important to determine the molecular basis of this modulation of the odontoblasts and to investigate whether molecular stimuli can act on different aspects of the cell's synthetic/secretory processes.

Molecular basis of odontoblast stimulation in reactionary dentinogenesis

Experiments on the implantation of isolated dentine ECM components reported above (Smith *et al.*, 1994) have demonstrated that interaction between these components and odontoblasts results in stimulation of the synthetic/secretory activity of these cells. Contact between odontoblasts and their extracellular matrix appears to be important in the maintenance of their phenotype as well. The phenotypic morphology of these cells when cultured can only be preserved as long as contact is maintained between the cells and dentine matrix (Munksgaard *et al.*, 1978; Heywood and Appleton, 1984). If the odontoblasts are removed from the tooth and cultured in isolation, they take on the appearance of a fibroblastic morphology. It is possible to replace the mature dentine matrix by preparations of isolated dentine ECM components as demonstrated by maintenance of odontoblast differentiation when dental papilla were cultured in contact with such preparations for 4 days (Lesot *et al.*, 1986).

The nature of the molecules in the isolated dentine ECM fractions which can interact with odontoblasts and stimulate their activity may be varied, although involvement of growth factors seems probable. The heparin-agarose bound material, which stimulated reactionary dentinogenesis as described in the implantation experiments above contained significant amounts of TGF-ß1, but not TGF-ß2 or 3. Culture of dental papillae with TGF-B1, or the closely related BMP-2, stimulated extracellular matrix secretion by these cells (Bègue-Kirn *et al.*, 1992). This pattern of cell behavior was different to that observed when these growth factors were added with potentiating molecules, such as heparin, when they were shown to be capable of inducing the differentiation of odontoblast-like cells (Bègue-Kirn *et al.*, 1992).

There are many reports of the effects of TGF-ß, and related members of this growth factor family, on matrix synthesis. BMP-2 has been reported to influence the activity of cultured osteoblastic MC3T3-E1 cells, where it stimulates alkaline phosphatase and collagen synthesis (Takuwa *et al.*, 1991). TGF-ß modulates the synthesis of collagens (Ignotz and Massagué, 1986; Yu *et al.*, 1991) and that of proteoglycans (Rapraeger, 1989; Yu *et al.*, 1991). Clearly, TGF-ß and related molecules can have a marked influence on extracellular matrix synthesis, but it is important to note that, depending on its concentration, TGF-ß can stimulate matrix synthesis or resorption (Tashjian *et al.*, 1985; Hock *et al.*, 1990). Thus, the control mechanisms on the activity of these growth factors is of paramount importance in determining their effects on cell behavior in the tissue.

There are a number of levels at which control of growth factor activity could occur within the tissue. At the level of interaction with the cell, whilst considerable information exists on TGF-B receptors (Boyde *et al.*, 1990) in general, little information exists on these receptors in relation to odontoblasts. The presence of the extracellular matrix proteoglycan, decorin, has been recently reported in dentine matrix (Lesot *et al.*, 1994). Decorin not only binds TGF-ß, but can also neutralize the activity of this growth factor. The time course for initiation of reactionary dentinogenesis when isolated dentine ECM components are implanted in unexposed cavities (Smith *et al.*, 1994) suggests that the interactions of the active molecule(s) occurs on the cell body rather than the process of the odontoblast. Further studies are now needed to elucidate the nature of the odontoblast growth factor interactions.

Control of growth factor activity can also occur by virtue of the availability of the growth factor. Finkelman *et al.* (1990) have demonstrated the presence of a number of growth factors in dentine, including TGF-ß, and have suggested that the response of odontoblasts to dental caries may be mediated by release of these growth factors from dentine during demineralization by bacterial acids. Our own studies have shown the presence of TGF-B1, 2 and 3 in dentine and that these molecules may be bound up in different extracellular matrix tissue compartments (Smith *et al.*, unpublished observations). A proportion of these molecules can be released by demineralization, but some appear to be tightly associated with the collagenous matrix and require collagenase digestion to release them. It is not clear yet as to whether these molecules are present in an active or latent state, but further investigations are now required to understand the role of these molecules and whether they participate in physiological tissue function (perhaps helping to maintain the odontoblast phenotype) or if they are only available/exposed in pathological conditions when they can initiate a response of tertiary dentinogenesis.

Conclusions

Deposition of reactionary dentine by post-mitotic odontoblasts, which have differentiated during tooth development, can arise at the pulp-dentine interface and at a peritubular/intratubular location in response to an appropriate stimulus. Some of this reactionary dentine may be a feature of normal physiological processes, such as aging, although it is not clear as to what extent pathological processes such as dental disease, tooth wear etc. contribute to this "physiological" appearance. Probably the majority of reactionary dentine arises in response to dental disease. Traditional views of the acid generated from plaque bacterial metabolism being the stimulus to the odontoblasts for reactionary dentinogenesis have little evidence to support them. It is likely that plaque bacterial acids solubilize growth factors, TGF-ß in particular, as they permeate dentine and that it is the solubilized growth factors that are responsible for initiating the stimulatory effect on the odontoblasts to secrete reactionary dentine.

Acknowledgments

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