The Future Role of a Molecular Approach to Pulp-Dentinal Regeneration

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Key Words

Dentin · Growth factors · Odontoblasts · Odontoblast-like cells · Pulp · Reactionary dentin · Reparative dentin · TGF- β · Vital pulp therapy

Abstract

The ultimate goal of a regenerative pulp treatment strategy is to reconstitute normal tissue continuum at the pulp-dentin border, regulating tissue-specific processes of tertiary dentinogenesis. Experimental investigations in mature teeth have shown that a network of extracellular matrix molecules and growth factors signal tertiary dentinogenesis. Application of dentin matrix components or growth factors in deep dentinal cavities stimulated up-regulation of biosynthetic activity of primary odontoblasts (reactionary dentin formation). Pulp-capping studies with a broad spectrum of biological agents, including growth factors and extracellular matrix molecules, showed formation of osteodentin and/or tertiary dentinogenesis (reparative dentin formation). Promising biologically active substances should be subjected to careful evaluation in well-designed preclinical investigations as well as in long-term clinical trials before their introduction in clinical practice.

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Vital pulp therapy aims to treat reversible pulpal injury and maintain pulp vitality and function. It includes two therapeutic approaches: indirect pulp capping in cases of deep dentinal cavities and direct pulp capping/pulpotomy in cases of pulp exposures. Successful outcome for vital pulp therapy is very dependent on the type and location of injury, age of the tooth, treatment modality (capping material) and integrity of the cavity restoration [for reviews, see Mjör, 2002; Horsted-Bindslev and Bergenholz, 2003]. This paper focuses on the potential therapeutic role of biologically active molecules as treatment modalities in vital therapy.

Whilst the biological processes directed by the treatment strategy have received much attention during the last four decades, controversy still exists regarding the biological basis of the mechanism by which the capping material regulates healing and repair of the pulp in vital pulp therapy [Nyborg, 1955; Fitzgerald, 1979; Cox et al., 1985; Horsted et al., 1985; Schroder, 1985; Cvek et al., 1987; Stanley, 1989; Mjör et al., 1991]. Advances in biomedical research open directions to design new methods of dental treatment, aiming at regeneration of the dentin-pulp complex. Numerous agents, delivering biologically active molecules at pulp exposure, were under investigation during the last decade. New approaches have been based on the understanding of the molecular and cellular mechanisms regulating dentinogenesis during dental tissue repair and their potential for clinical exploitation.

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Tertiary Dentinogenesis in Vital Pulp Therapy

The dental pulp possesses the ability to form a dentin-like matrix (tertiary dentin) as a part of repair in the dentin-pulp organ [Baume, 1980]. Vital pulp therapy aims to treat reversible pulpal injury, whenever dentin and pulp are affected by caries, restorative procedures or trauma. The injury may or may not involve pulpal exposure and is followed by a classical wound healing process of the connective tissue. Wound healing and new hard tissue formation beneath the injury are prerequisites for long-term control of post-operative infection and pulp survival in vital pulp therapy.

It is well recognized that the nature and specificity by which a traumatized tissue area is healed determine the biological properties of the newly formed tissues. The end result of the healing process in vital pulp therapy would be a reconstitution of normal tissue architecture and tertiary dentin formation at the wound area, or formation of scar-like soft tissue and fibrodentin formation. The healing pattern may be dependent, partly at least, on the type and extent of tissue injury and the effect of the associated defence reaction on the structural and functional integrity at the dentin-pulp border [for a review, see Smith, 2002]. Principally, whenever the dentin-pulp complex is affected by injury, three different physio-pathological conditions could be observed at the dentin-pulp border.

(a) In the case of mild injuries, odontoblasts may survive, e.g. non-cavitated stages of enamel caries, slowly progressing dentinal caries, mild abrasion, erosion, mechanico-chemical irritation or fracture involving enamel-dentin. The odontoblast layer is stimulated to form tertiary dentin matrix beneath the injury (reactionary dentin), while peritubular dentin formation is seen in the dentinal tubules [Frank, 1968; Stanley et al., 1983; Bjørndal and Mjör, 2001; Murray and Smith, 2002]. Reactionary dentin shows many anatomical, biochemical and functional similarities to the primary and secondary dentin and can effectively oppose exogenous destructive stimuli to protect the pulp [Smith et al., 2002]. Reactionary dentinogenesis represents up-regulation of the biosynthetic activity of primary odontoblasts, restricted to those cells affected by the injury. Peritubular dentin formation should be distinguished from atypical intratubular calcification, which has been suggested to represent a non-vital process [Frank, 1968].

(b) With severe dentinal injuries without pulp exposure, odontoblasts are destroyed subjacent to the affected dentin, e.g. rapidly progressive carious lesions, severe tissue damage due to cavity preparation or cytotoxic injury

on pulpal cells due to restoration [Stanley et al., 1983; Kitamura et al., 2001; Bjørndal and Mjör, 2001]. A cascade of inflammatory and healing events rapidly occurs in the area of degenerating odontoblasts [Kim, 1990; Chiego, 1992]. As a part of the connective tissue healing, pulpal cells proliferate and migrate toward the circumpulpal dentin. Initially, fibroblast-like cells align themselves against the dentin and atubular fibrodentin is laid down at the dentin-pulp border. In an appropriate metabolic state of the dentin-pulp complex, a new generation of odontoblast-like cells may differentiate and form tubular tertiary dentin (reparative dentinogenesis) in a polar predentin-like pattern [Baume, 1980; Bjørndal and Darvann, 1999]. It must be emphasized that under clinical conditions matrix formed at the pulp-dentin interface often comprises reactionary dentin, reparative dentin or fibrodentin formation. It is impossible to distinguish these processes at the *in vivo* level and the processes may also from a biochemical and molecular point of view be indistinguishable.

(c) In the case of pulp exposure, the amputated pulp can be repaired by itself or after application of capping materials [Nyborg, 1955; Kakehashi et al., 1965; Yamamura, 1985]. Pulpal exposure due to caries shows very limited potential for pulp recovery due to bacterial infection of the pulp for a substantial period of time, which compromises the defence reaction [Bergenholtz, 2001]. Favourable conditions for pulp repair after oral exposure require an environment free of bacteria, absence of severe haemodynamic changes and absence of severe inflammatory cell infiltration. Whether subsequent reactions lead to pulp healing and repair or to generalized pulp inflammation and necrosis will depend on the extent of defence reactions [Trowbridge, 1981]. As a part of the wound healing process in the repairing pulp, the dentinogenic potential of pulpal cells can be expressed [for a review, see Tziafas, 1997]. Proliferation, migration and differentiation of progenitor cells can give rise to a new generation of reparative dentin-forming cells (odontoblast-like cells) reconstituting the lost continuum at the pulp-dentin border [Fitzgerald, 1979; Fitzgerald et al., 1990; Mjör et al., 1991].

The cellular processes taking place after pulp exposure have been elucidated by using calcium hydroxide-based materials or other materials producing a low-grade irritation to the pulp. Initially, the pulpal cells under the capping material proliferate, migrate and elaborate new collagen in contact with a firm necrotic zone of the treated pulpal area. Then, mineral salts precipitate on the necrotic zone and in the associated new collagen matrix. Finally,

Fig. 1. Transdental stimulation of reactionary dentinogenesis in the case of mild dentinal injury. The ultimate goal of a regenerative treatment strategy is to up-regulate the biosynthetic activity of survived primary odontoblasts corresponding to the involved area.

reparative dentinogenesis is initiated; a layer of odontoblast-like cells is formed in association with the superficial calcification and a tubular mineralized matrix is secreted in a polar predentin-like pattern [Schroder, 1985; Cvek, 1987]. However, many studies have shown that the wound healing mechanism often results in early formation of fibrodentin with osteotypic appearance at the traumatized area [Baume, 1980; Cox et al., 1996; Higashi and Okamoto, 1996]. Osteotypic hard tissue cannot provide the necessary barrier effect to protect the pulp from exogenous destructive stimuli.

Therapeutic Regulation of Tertiary Dentinogenesis: Existing Knowledge and Future Perspectives for Research

The ability of the pulp-dentin complex to respond to therapeutic applications by specific cellular processes and hard tissue formation has long been recognized. Current research has provided insights into the basic molecular events underlying dental tissue repair, induction of tertiary dentin formation, competence of the responsive cells and how these phenomena could be integrated into the clinical approach to the problem of vital pulp therapy [for reviews, see Lesot et al., 1994; Smith et al., 1995; Rutherford, 1999; Tziafas et al., 2000].

Transdental Stimulation of Reactionary Dentinogenesis

The aim of a regenerative treatment strategy in the case of mild dentinal injuries (fig. 1) is to stimulate localized peritubular dentin formation and to provide a regional and time-limited effect on surviving odontoblasts, in order to up-regulate their biosynthetic activity. The optimal end result is sclerosis of primary dentin and an

underlying zone of reactionary dentin at the pulp-dentin border corresponding to the involved area.

The molecular processes underlying reactionary dentinogenesis have recently been approached in experimental models in vitro and in vivo and in carious human teeth [Magloire et al., 1996; Sloan and Smith, 1999]. Our understanding in this field has significantly advanced with appreciation that dentin extracellular matrix does not represent an inert material, but it contains bioactive molecules potentially available for release during pulp healing and repair [Finkelman et al., 1991; Magloire et al., 2001; Smith et al., 2001]. Carious demineralization of the dentin has been suggested to release bioactive molecules, which signal the associated dentinogenic events [Smith, 2002]. Dental materials may also solubilize and release growth factors (TGF- β s) from the soluble fraction of dentin components [Zhao et al., 2000]. Application of EDTA-soluble dentin components in unexposed cavities of ferret teeth demonstrated stimulation of odontoblasts forming a zone of reactionary dentin beneath the injury [Smith et al., 1994, 2001]. Transdental stimulation of tertiary dentinogenesis was also seen after application of osteogenic protein-1 (bone morphogenetic protein-7) in unexposed cavities of monkey teeth [Rutherford et al., 1995]. In unexposed deep cavities of dog teeth, human recombinant TGF- β 1 was placed on acid-treated dentin. A specific effect of TGF- β 1 producing intratubular mineralization in a superficial zone of the treated dentin was seen [unpubl. data]. Further studies are still required to fully understand the kinetics of growth factors and the associated stimulation of dentinogenic events.

Transdental Stimulation of Reparative Dentinogenesis

A regenerative therapeutic approach, in the case of severe localized injury without pulp exposure (fig. 2), may

Fig. 2. Transdentinal stimulation of reparative dentinogenesis in the case of severe dentinal injury. The ultimate goal of a regenerative treatment strategy is to favour the biological activity of dentin matrix, which in an appropriate pulpal environment is able to trigger differentiation of new odontoblast-like cells replacing lost primary odontoblasts.

result in differentiation of odontoblast-like cells for replacement of the lost odontoblasts and a time-limited formation of reparative dentin corresponding to the involved area.

The debate on the origin of odontoblast-like cells and the associated signaling mechanisms controlling migration, orientation, attachment and cytodifferentiation of pulpal cells remains to be resolved. It has been strongly suggested that the exposed dentinal surface and the accumulated bioactive molecules might provide the necessary signals that determine the underlying cell function [Heritier et al., 1990]. Implantation of autogenous demineralized, or native, or unmineralized dentin matrices into the pulp at a distance from the site of mechanical pulp exposure allowed us to study potential interactions between dentinal matrix and the pulpal cells [Tziafas et al., 1992]. It is evident that the normal sequence of reparative events do not take place in the intrapulpal test model, but the biological effects of exogenous matrices or molecules on pulpal cells with minimal tissue trauma can be evaluated. In close proximity to demineralized dentin, we found stimulated spindle-shaped or polygonal cells after 3 days, groups of cells undergoing differentiation in relation to a newly formed matrix after 7 days and mineralized reparative dentin with a new layer of odontoblast-like cells after 2 weeks. The response of the pulpal cells to demineralized dentin was also characterized by deposition of fibrodentinal matrix before initiation of reparative dentinogenesis. Direct odontoblast-like cell differentiation in close proximity to the implanted unmineralized dentin matrix (predentin) was seen 3 days after implantation. These data indicate two mechanisms for reparative dentinogenesis onto the dentin surface: direct induction of odontoblast-like cells by the dentin matrix or indirect differentiation of odontoblast-like cells on an intermediate fibrodentinal matrix. Hence, predentin surface seems to represent

an appropriate substratum for direct induction of reparative dentin. Already in 1985, Mjör [1985] reported early formation of atubular fibrodentin (or interface dentin) before the onset of tertiary dentin formation after localized odontoblast destruction in deep dentinal cavities.

The dentinogenic activity of dentin matrix might be attributed to the soluble fraction of dentin components. Morphological and functional differentiation of odontoblast-like cells was seen in close proximity to Millipore filters containing EDTA-soluble dentin components after 8 days [Tziafas et al., 1995]. The dentinogenic activity of this dentin fraction had previously been demonstrated in vitro [Begue-Kirn et al., 1992] and it was shown that the dentinogenic activity could be abolished by preincubation of the components with an antibody blocking the biological activity of TGF- β molecules. Similarly, any effect of antibody-treated dentin on pulpal cells was completely or partially lost in vivo, indicating that the dentinogenic activity of dentin matrix can at least partly be ascribed to the TGF- β molecules [Tziafas, 1995]. It seems that the endogenous pools of TGF- β s and other growth factors in the dentin matrix may provide a natural delivery system, regulating reparative dentinogenesis after destruction of primary odontoblasts in deep dentinal cavities. Further studies are required to clarify whether the biological effect of dentin matrix on initiation of reparative dentinogenesis could be triggered therapeutically.

Direct Induction of Reparative Dentinogenesis

The ultimate goal of a regenerative treatment strategy in direct pulp capping or pulpotomy situation is to induce differentiation of odontoblast-like cells forming reparative dentin at the pulp-capping material interface (fig. 3) and to stimulate the biosynthetic activity of surrounding primary odontoblasts [Mjör, 2002]. The optimal end result is the reconstitution of dentinal defect with a bridge

Fig. 3. Direct induction of reparative dentinogenesis in pulp exposure. The ultimate goal of a regenerative treatment strategy is to induce differentiation of odontoblast-like cells at the pulp-capping material interface and to up-regulate the biosynthetic activity of primary odontoblasts around the pulpal exposure to reconstitute the lost continuum of the pulp-dentin border.

of reparative dentin in direct continuum with reactionary dentin formed around the pulp exposure.

The signaling mechanisms regulating reparative dentinogenesis after pulp capping have not been fully understood. The nature of the pulp wound healing mechanism depends on the defence reaction, possible contamination with oral bacteria, bleeding during surgery or cramming of dentinal chips into the pulp space [Heys et al., 1981, 1990; Cvek et al., 1987; Cox et al., 1987; Stanley, 1989]. It has been postulated that a network of interactions between extracellular matrix molecules, including fibronectin [Yoshida et al., 1996; Tziafas et al., 1995], and growth factors regulates odontoblast-like cell differentiation and reparative dentinogenesis in the repairing pulp environment [Lesot et al., 1994]. The presence of a mechanical support seems to be of critical importance. Intermediate fibrodentinal matrix may act as the basement membrane does for odontoblast differentiation during tooth formation [Ruch, 1985].

In numerous animal studies, application of biologically active growth and morphogenetic factors and extracellular matrix molecules as capping materials resulted in hard tissue formation. Bone morphogenetic proteins (BMP), such as BMP-2, BMP-4 and BMP-7 (osteogenic protein-1), induced formation of osteodentin in large amounts followed by tubular reparative dentin [Nakashima, 1994a, b; Rutherford et al., 1993; Jepsen et al., 1997]. Capping experiments with insulin-like growth factor-I have demonstrated complete dentinal bridging and occasionally tubular reparative dentin formation [Lovschall et al., 2001]. Osteodentin followed by homogeneous and well-mineralized atubular reparative dentin was seen after capping treatment with bone sialoprotein [Decup et al., 2000]. Hard tissue formation at a distance from the capping was found after placement of enamel matrix derivatives in the exposed pulp [Nakamura et al., 2001].

Intrapulpal implantation of Millipore filters containing either EDTA-soluble dentin constituents [Tziafas et al., 1995] or human TGF- β 1 [Tziafas et al., 1998] induced specific dentinogenic events in close proximity to the implants. Cytological differentiation of odontoblast-like cells and reparative dentin formation were seen around the filters. Implantation of Millipore filters containing other growth factors, such as basic fibroblast growth factor or insulin-like growth factor-II, showed increased dentinogenic effect at a distance from the implant [Tziafas et al., 1998]. It seems that while TGF- β 1 appears to be an effective signal for odontoblast-like cell differentiation within the pulp, its ability to do so at the wound surface is very limited. Nakashima [1994b] showed inhibition of reparative dentin formation after pulp capping with collagen containing TGF- β 1. Experimental applications of several artificial substrates, such as Millipore filters, hydroxyapatite granules, pure titanium, as carriers for recombinant human TGF- β 1 used for pulp capping in dog teeth failed to induce any dentinogenic effect. Only pre-set calcium hydroxide soaked in recombinant human TGF- β 1 stimulated differentiation of odontoblast-like cells and reparative dentin, while the control teeth, which were capped with pre-set calcium hydroxide only, did not show any particular response [Tziafas et al., 2001].

It is clear that a broad spectrum of biological substances stimulate reparative dentin formation in the exposed pulp. However, it is important to recognize that in most of these cases the reparative dentinogenesis proceeded via formation of fibrodentin matrix. As has been previously stated [Tziafas et al., 2000], formation of fibrodentin perhaps implies an indirect effect, e.g. stimulation of the biosynthetic activity of pulpal cells, which is later superseded by the tissue-specific dentinogenic response. The clinical problem with the indirect effects of biologically active molecules on pulpal cells is that fibro-

dentin formation does not represent a guided natural regeneration at the dentin-pulp border.

Development of new capping materials for delivery of exogenous signaling molecules offers exciting opportunities for the future. However, a number of critical considerations, such as the dose-response effects, the nature of the delivery system, half-life of the molecules and their possible side-effects need to be addressed before any introduction of new treatment modalities into clinical practice.

Modern materials able to exploit endogenous biologically active molecules could also be used in the shorter-term at least. In any case, promising new treatment strategies should be exposed to careful evaluation in properly designed preclinical investigations with a large number of capping experiments and in well-designed clinical trials to account all possible variables that may exist clinically [Bergenholtz, 2001].

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