Oral Diseases (2004) 10, 63–74

http://www.blackwellmunksgaard.com

REVIEW
Oral Biology

Dentin matrix proteins and soluble factors: intrinsic regulatory signals for healing and resorption of dental and periodontal tissues?

TA Silva[1], AL Rosa[2], VS Lara[1]

[1]*Department of Stomatology, Faculty of Dentistry of Bauru and* [2]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry of Ribeirão Preto, University of São Paulo, São Paulo, Brazil*

Dentin contains numerous polypeptides and signaling molecules sequestered in a mineralized matrix. The exposure and release of these molecules occur as a consequence of injury to the pulp and periodontal ligament, which may result from luxation, orthodontic movement or infections of tooth and periodontal structures. When released at these sites, dentin constituents have the potential to act on different surrounding cells, including periodontal cells, osteoblasts, osteoclasts and inflammatory cells, and to affect the course of dental disease. Experimental studies have highlighted the interactions between dentin and cells from tooth and periodontal tissues and reveal dentin to be a cell adhesive, signaling and migratory stimulus for various mesenchymal and inflammatory cells. These results support the hypothesis that dentin molecules might function as regulatory signals for the healing and resorption of dental and periodontal tissues. Data from recent and classical investigations are summarized, many open questions are discussed, and current hypotheses concerning the mechanisms of tooth resorption and periodontal healing are outlined. Many questions regarding the importance of dentin as a source of multifunctional molecules remain unanswered and provide important directions for future studies.
Oral Diseases (2004) **10**, 63–74

Keywords: dentin; periodontal healing; dental resorption; bone cells; cytokines; bone resorption

Correspondence: Tarcília Aparecida da Silva, Department of Stomatology (Pathology), Faculty of Dentistry of Bauru, University of São Paulo, Rua Flor de Fogo 65, apto 404, Bloco 6, 31270-217 – Belo Horizonte, Minas Gerais, Brazil. Tel: +55 31 3427 6530, Fax: +55 6332301, E-mail: tarcilia@zipmail.com.br
Received 5 February 2003; revised 12 May 2003 and 24 September 2003; accepted 17 November 2003

Introduction

Odontoblasts secrete dentin organic components, which include a complex mixture of proteoglycans, glycoproteins, sialoproteins, phosphoproteins and a variety of other molecules (Linde, 1989; Butler *et al*, 1992; Butler, 1998). During dentinogenesis the extracellular matrix of dentin is mineralized by deposition of hydroxyapatite onto the fibrous matrix. After mineralization the dentin molecules remain trapped in the mineralized phase bound to matrix components or to hydroxyapatite crystals, which may be exposed or released as a consequence of injury to the periodontal ligament (PDL) and dental pulp by, for example, trauma, periodontal disease and orthodontic tooth movement (Ne, Whiterspoon and Gutmann, 1999). Critically, there is considerable scope for the involvement of dentin, if released, in processes that affect surrounding tissues (Finkelman, 1992; Smith and Lesot, 2001). The physiological significance of potentially active molecules sequestered in dentin has not been fully investigated; however, some results indicate that dentin triggers the migration and activation of several cell types and interferes with the processes of resorption or repair through this mechanism, in a similar way to the bone remodeling process. Several growth factors and their receptors, which are expressed during embryonic tooth development, remain sequestered in mineralized tissues and are also re-expressed in mature dental and periodontal tissues under normal and pathological conditions (Matsuda *et al*, 1993; Booth *et al*, 1998; Takayama *et al*, 1998; Johnson, Serio and Dai, 1999; Steinsvoll, Halstensen and Schenck, 1999; Ivanovski, Haase and Bartold, 2001; King and Hughes, 2001; Parkar *et al*, 2001). In pathological conditions, cementoblasts, osteoblasts, fibroblasts and other cells are often lost and then replaced by undifferentiated cells. These cells are able to differentiate and produce reparative tissues through the action of signaling molecules in this

microenvironment. These regulatory mechanisms use multiple cytokines, which act in concert with numerous other factors, including cell–cell interactions, extracellular matrix molecules, and levels of differentiation. Local conditions, such as trauma and infections may disrupt the physiologic balance in bone and dental tissues by interfering with the availability of growth factors and signaling proteins from dentin and periodontal cells.

Some *in vitro* experiments have shown that the surface of dentin is an excellent substratum for cell adhesion (Tenorio, Foyle and Hughes, 1997; Scheven, Marshall and Aspden, 2002). However, the exposure of dentin *in vivo* allows osteoclasts to colonize the root surface and start the resorption process. These cells dissolve the mineralized matrix, then endocytose, transport and continually release the matrix components during the resorption process (Nesbitt and Horton, 1997). Once released, these molecules might be able to act at resorption sites and function as chemotactic and activator signals for osteoblasts (Ogata *et al*, 1997; Tenorio *et al*, 1997; Takata *et al*, 1998), PDL cells (Somerman *et al*, 1987a,b; Ogata *et al*, 1997), neutrophils and macrophages (Lara *et al*, 2003) and, theoretically, influence the course of root resorption.

Although dentin has been considered to have the potential to alter cell function within the tooth/periodontal microenvironment, the underlying mechanisms are still poorly understood, especially regarding how these cells interact with dental hard tissues and if the release of dentin molecules contributes toward maintaining resorption or inducing repair during pathological exposure. In addition, it remains unknown whether functional molecules could be released after demineralization and if there are sufficient amounts of active molecules to contribute significantly to the regulation of resorption and repair. Considerable experimental effort has been expended in order to identify the mediator(s) responsible for the observed effects of dentin matrix. The results concerning the *in vitro* and *in vivo* responsiveness to dentin appear to be contradictory, but the differences may be explained by the use of different culture systems, methods for dentin extraction, sources of dentin and methods of cell treatments. The purpose of this article is to review the literature with respect to the effects of dentin on several cell types and tissues and to present hypotheses that integrate this information into the context of tooth resorption and periodontal healing mechanisms.

Potential bioactive dentin molecules

A number of non-collagenous proteins have been isolated from dentin and bone tissue and they exhibit qualitative similarities but significant quantitative differences. Most notable among these differences are the low levels of dentin sialoprotein and dentin phosphoprotein in bone and the low amounts of osteopontin (OPN) in dentin (Fujisawa *et al*, 1993; Qin *et al*, 2002). The functions of these proteins remain to be definitively established (Butler *et al*, 1992; Butler, 1998; Qin *et al*, 2002).

The majority of molecules that can be chemically extracted from dentin reflect their formative roles in the dentinogenesis process. Recently, a variety of molecules including cytokines and enzymes have been found in dentin but little is known about their function in this tissue. The cytokines, a group, which includes growth factors, are small soluble proteins capable of altering the behavior of other cells locally or systemically.

Similar to bone, dentin represents a significant storage site for insulin-like growth factor (IGF)-I, IGF-II, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), placenta growth factor (PlGF), fibroblast growth factor (FGF), metalloproteinase (MMP) and transforming growth factor (TGF)-β super family members such as TGF-β (Table 1) (Finkelman *et al*, 1990; Cassidy *et al*, 1997; Thomadakis *et al*, 1999; Heras, Valenzuela and Overall, 2000; Roberts-Clark and Smith, 2000; Veis *et al*, 2000). As dentin normally does not undergo remodeling in the way that bone does, the identification of cytokines and enzymes in dentin raises the question of the function of these molecules. In order to explore these findings, in the following sections we focus mainly on the biologic activity of dentin molecules in the context of pathological processes affecting dental and periodontal tissues.

Cellular responsiveness to dentin

Dentin osteoinductive properties

Dentin extracellular matrix has been shown to contain components capable of inducing chondrogenesis and osteogenesis at ectopic sites when implanted *in vivo*, and chondrogenesis in cultures of embryonic muscle-derived fibroblasts (EMF) *in vitro* (Huggins, Wiseman and Reddi, 1970). Studies in which blocks, rolls, slices or powder of demineralized dentin were implanted into bone defects in humans and animals provided convincing evidence that dentin induces bone formation (Bang and Urist, 1967; Yoemans and Urist, 1967).

Induction of chondrogenesis *in vivo* by rolls of demineralized dentin implanted into a number of tissues occurs initially in muscle, followed by subcutaneous connective tissue of skin and medullary cavity of femur, and later in PDL (Inoue, Deporter and Melcher, 1986). Significantly, more cartilage was found in muscle than in subcutaneous connective tissue of skin and medullary cavity of femur. Outgrowth of cells from rat muscle, dermis and subcutaneous tissue, bone marrow and PDL cultured *in vitro* on demineralized bone matrix produced similar results (Inoue, Chen and Shimono, 1990; Rabinowitz, Syftestad and Caplan, 1990).

In order to understand the mechanisms of dentin-induced osteogenesis, many researchers investigated the molecules embedded within the dentin or bone matrix responsible for this effect. While it is important to note that numerous dentin constituents that are described in the following sections, together with as yet unidentified

Table 1 Growth factors and other soluble molecules identified in dentin

Type	*Species*	*Concentration*	*Reference*
IGF-I	Human	0.06 ng μg^{-1} G-EDTA	Finkelman *et al* (1990)
SGF/IGFII		0.52 ng μg^{-1} G-EDTA	
TGF-β		0.017 ng μg^{-1} G-EDTA	
TGF-β1	Human	166–288 pg mg^{-1}	Cassidy *et al* (1997)
TGF-β2		NT	
TGF-β3		NT	
TGF-β1	Rabbit	439–685 pg mg^{-1}	
TGF-β2		19 pg mg^{-1}	
TGF-β3		45–64 pg mg^{-1}	
TGF-β	Bovine	6.6 ng mg^{-1}	Takata *et al* (1998)
MMP gelatinase A	Human	ND	Heras *et al* (2000)
PDGF-AB	Human	570 ± 42.8 pg mg^{-1}	Roberts-Clark and Smith (2000)
VEGF		63 ± 2.3 pg mg^{-1}	
PlGF		24 ± 1.4 pg mg^{-1}	
FGF2		40 ± 0.9 pg mg^{-1}	
EGF		1 ± 0.04 pg mg^{-1}	
[A + 4][a]	Rat	ND	Veis *et al* (2000)
[A4][a]		ND	

[a]Amelogenin gene splice products. ND, not determined; NT, not detected.

substances, may be involved in dentin-induced osteogenesis the principle component that induces chondrogenesis and osteogenic transformation has been termed bone morphogenetic protein (BMP) (Urist and Strates, 1971). BMPs are soluble, locally acting signaling molecules, which bind to specific receptors on the surface of cells. These receptors activate signal transduction mechanisms leading to the induction of particular genes. *In vivo*, BMPs act primarily as differentiation factors, turning responsive mesenchymal cells into cartilage- and bone-forming cells. The bone induction is only observed locally at the site of experimental implantation and it is limited temporally to the period when the BMP is present. While BMP induces bone formation *in vivo*, *in vitro* only cartilage production was observed. These findings might indicate that BMP is involved in the differentiation into cartilage of immature mesenchymal-type cells, but the subsequent process of endochondral bone differentiation is regulated by other growth factors. Moreover, these differences may be only a result of differences between *in vitro* and *in vivo* models. The dentin matrix has a higher level of BMP activity than bone (Urist and Strates, 1971; Veis, Sires and Clohisy, 1989). The purification and characterization of BMP were difficult because of the very small amounts present and its interaction with other matrix components. However, BMP has been purified from dental hard tissues including dentin, enamel and cementum (Kawai and Urist, 1989; Bessho, Tagawa and Murata, 1990; Bessho *et al*, 1991) and a number of BMP products are now available for clinical use (Hoffmann *et al*, 2001; King and Cochran, 2002).

Although its potential *in vivo* function remains elusive some studies have demonstrated the presence in dentin of a unique molecule which has the ability to induce EMF, *in vitro*, to increase the synthesis of sulfated proteoglycans, to produce type II collagen and to undergo morphodifferentiation in which nodules of rounded cells are formed surrounded by a type II collagen and a proteoglycan-rich extracellular matrix (Veis *et al*, 1989, 1990).

Veis *et al* (1989) first attempted to isolate the matrix factor responsible for initiating osteogenesis from rat incisor dentin. The polypeptide responsible for dentin-induced chondrogenesis, called chondrogenic-inducing agent (CIA), has been isolated from a 4.0-M guanidinium hydrochloride extract of demineralized bovine dentin matrix. CIA activity was identified in fractions by assay for uptake of [35S]-SO_4 into proteoglycan by the EMF. *In vivo* implants in rat muscle with collagen carrier produced ectopic bone after 7 weeks. The CIA components had masses in the ranges of 6–10 kDa and amino acid composition, NH_2-terminal, and internal amino acid sequences, which revealed homology between CIA peptides and bovine amelogenin (Nebgen *et al*, 1999). It was postulated that during tooth development these specific amelogenin gene splice products, [A + 4] and [A4], may have a role in preodontoblast maturation. The [A + 4] and [A4] may thus be tissue-specific epithelial mesenchymal signaling molecules (Veis *et al*, 2000). In fact, the receptors for the small amelogenin gene splice product [A-4] were shown to be present on the cell surface of embryonic mesenchymal fibroblasts (Tompkins and Veis, 2001). It is not apparent why dentin should contain such activity considering that it neither follows a chondrogenic pathway during dentinogenesis nor remodels as bone does. One factor is that dentinogenesis depends on a specific temporal and spatial cascade of epithelio–mesenchymal interactions during which epigenetic signals determine the pathways of differentiation. In this way, the CIA polypeptide may be involved in some aspects of this process. It is of interest to speculate why chondrostimulatory substances comprise a fraction of non-collagenous proteins of dentin and if these substances could play a role in the repair of periodontal tissues

given that the induction of cartilage and subsequent endochondral osteogenesis could be viewed as a modification of the healing process.

Effects of dentin and its constituents on periodontal cells
A number of studies have focused on the migration of periodontal cells in response to chemotactic factors from cementum, dentin and bone suggesting that such factors could regulate the migration and orientation of these cells during periodontal wound healing (Miki, Narayanan and Page, 1987; Somerman *et al*, 1987a,b, 1989). Several macromolecules merit further study concerning healing of dental and periodontal tissues (see Table 2). Extracts from cementum, bone, dentin and enamel were compared concerning the *in vitro* chemotactic behavior of human PDL cells, human gingival fibroblasts and human alveolar bone cells. The chemotactic effects of all extracts were reduced by heat- and trypsin-treatment but not affected by treatment with anti-fibronectin antibody. Other molecules such as TGF-β, EGF, PDGF, and osteocalcin (OC) were ruled out as chemotactic factors from dental hard tissues (Nishimura *et al*, 1989; Ogata *et al*, 1997).

Slices of dentin, cementum and bone were found to modulate the function of osteoblastic cells through induction of 'acellular cementum-like' deposition and increase in alkaline phosphatase activity (Tenorio *et al*, 1997; Scheven *et al*, 2002). Alternatively, the effect of

Table 2 Summary of reported effects of dentin molecules on periodontal and bone tissues

Soluble factors	*Effects on periodontal cells and tissues (reference)*	*Effects on bone cells activity (reference)*
TGF-β	(+) Cementoblast phenotype (Saygin *et al*, 2000)	(−) OCL formation (Chenu *et al*, 1988) (+) Bone formation (Noda and Camilliere, 1989) (+) Bone resorption (Tsahjian *et al*, 1985) (−) Bone resorption (Berghuis *et al*, 1994) (−) OBL proliferation (Takata *et al*, 1998) (±) OBL phenotype (Locklin *et al*, 1999)
BMPs	(+) Cementogenesis (Ripamonti *et al*, 1996; King and Hughes, 2001) (−) Cementoblast mineralization (Zhao *et al*, 2003)	(+) Bone formation (King and Cochran, 2002) (+) OBL differentiation (Yamaguchi *et al*, 2000) (+) Osteoclastogenesis (Koide *et al*, 1999) (±) Bone resorption (Kanatani *et al*, 1995) (+) Bone resorption (Hentunen *et al*, 1995)
VEGF	(?)	(+) Bone resorption (Nakagawa *et al*, 2000) (+) Osteoclastogenesis (Niida *et al*, 1999)
EGF	(−) Cementoblast phenotype (Matsuda *et al*, 1993)	(+) Bone resorption (Tsahjian and Levine, 1978)
IGF	(+) Cementoblast phenotype (Saygin *et al*, 2000) (+) PDL cells chemotaxis-proliferation (Matsuda *et al*, 1992)	(+) Bone formation (Hock *et al*, 1988) (+) Bone resorption (Hill *et al*, 1995) (+) OCL recruitment and activation (Mochizuki *et al*, 1992)
PDGF	(+) Cementoblast phenotype (Saygin *et al*, 2000) (+) PDL cells proliferation (Matsuda *et al*, 1992; Mumford *et al*, 2001)	(+) Bone resorption (Tashjian *et al*, 1982; Hill *et al*, 1995) (+) OCL recruitment/activation (Mochizuki *et al*, 1992)
FGF	(+) PDL cells chemotaxis and proliferation (Terranova *et al*, 1989; Murakami *et al*, 1999)	(+) Bone formation (Dunstan *et al*, 1999) (+) Bone resorption (Kawaguchi *et al*, 2000; Collin-Osdoby *et al*, 2002) (+) Osteoclastogenesis (Hurley *et al*, 1998; Nakagawa *et al*, 1999)
BSP	(?)	(−) Bone resorption (Sato *et al*, 1992) (+) Bone resorption (Raynal *et al*, 1996; Razzouk *et al*, 2002) (+) OBL proliferation and differentiation (Zhou *et al*, 1995)
OC	(?)	(−) Bone formation (Ducy *et al*, 1996) (+) Monocytes and OC chemotaxis (Malone *et al*, 1982; Chenu *et al*, 1994) (+) Osteoclastogenesis (Glowacki *et al*, 1991; Liggett *et al*, 1994)
α2HS glycoprotein	(?)	(+) Monocytes chemotaxis (Malone *et al*, 1982)
BAG 75	(?)	(−) Bone resorption (Sato *et al*, 1992)
OPN	(?)	(−) Bone resorption (Sato *et al*, 1992) (+) Bone resorption (Razzouk *et al*, 2002)
MMP	(?)	(−) Bone resorption (Zhao *et al*, 1999)

(+) stimulation; (−) inhibition; (?) no study; OCL, osteoclasts; OBL, osteoblasts; PDL, periodontal ligament.

different dentin surface preparation techniques on osteoblast proliferation has been evaluated. Data from Schwartz *et al* (2000) indicate that demineralization of the dentin surface promotes proliferation of osteoblasts and early differentiation events including production of both alkaline phosphatase and autocrine mediators such as prostaglandins and TGF-β. However, later differentiation events such as OC production are decreased.

Guanidine-EDTA (G-EDTA) protein extracts of dentin had no effect on cell attachment or spreading in human gingival fibroblasts and PDL cells. These findings contrast with that for G-EDTA protein extracts of alveolar bone and cementum: all cementum extracts elicited significant increases in total protein production when compared with controls (Somerman *et al*, 1987a,b, 1989). Analogous results were obtained by Miki *et al* (1987), who analyzed the mitogenic activity of cementum and dentin extracts on human fibroblasts. They observed that cementum extracts, but not dentin, stimulated quiescent gingival fibroblasts to synthesize DNA.

Several effects of dentin on periodontal cells have also been attributed to molecules of the BMP family, which were shown to have the potential to stimulate regeneration of periodontium when implanted in periodontal defects (Ripamonti *et al*, 1996; King and Hughes, 2001; King and Cochran, 2002). Periodontal fibroblast-like cells incubated with exogenous BMPs showed decreased alkaline phosphatase activity and unchanged fibronectin production, but increased expression of endogenous BMPs compared with control specimens. This suggests that regenerative as well as inductive effects of BMPs on undifferentiated periodontal mesenchymal cells may be mediated through their ability to induce the expression of these molecules (Gao *et al*, 1995). In contrast, recent *in vitro* results show that BMP-2 inhibits the expression of genes related to mineralization in mature cementoblasts (Zhao, Berry and Somerman, 2003).

Knowledge of the responsiveness of cells within the periodontal region to specific bioactive agents is important for improving regenerative therapies. It has been observed that IGF-I, PDGF, and TGF-β influence mitogenesis, phenotypic gene expression profile, and biomineralization of cementoblasts (Matsuda *et al*, 1992; Saygin *et al*, 2000; Mumford *et al*, 2001), which may offer candidates for the regeneration of periodontal tissues. Periodontal research shows that FGF also provides a potent stimulus to periodontal wound healing by inducing the growth of immature PDL cells and promoting angiogenesis (Terranova *et al*, 1989; Murakami *et al*, 1999). Regarding the role of EGF in periodontal tissues, Matsuda *et al* (1993) showed that PDL fibroblasts express numerous EGF receptors that decrease in number as the cells differentiate into types capable of forming mineralized tissues under the influence of dexamethasone. From these results, they suggested that EGF might function in the stabilization of phenotype in PDL fibroblasts.

Besides dentin, epithelial cell rests (Hasegawa *et al*, 2003), as well as normal, regenerating and inflamed periodontal tissues (Booth *et al*, 1998; Takayama *et al*, 1998; Johnson *et al*, 1999; Steinsvoll *et al*, 1999; Ivanovski *et al*, 2001; King and Hughes, 2001) could act as sources of signaling molecules during periodontal repair. These factors will act on dental and periodontal cells through interaction with their cognate cell surface receptors (Takayama *et al*, 1998; Parkar *et al*, 2001). Against this background of growth factor functions in repair, dentin has been found to contain MMPs (Heras *et al*, 2000) and mature odontoblasts secrete MMP-2, MMP-9, and MMP-8 *in vitro* (Tjäderhane *et al*, 1998; Palosaari *et al*, 2000). These enzymes are part of a family of proteinases that are capable of cleaving virtually all of the components of the extracellular matrix and may regulate the availability of signaling molecules from dentin and periodontal tissues. Here, as elsewhere in the body MMPs activity may be controlled by tissue inhibitors of metalloproteinases as these enzymes are found to be concomitantly expressed in periodontal tissues (Ejeil *et al*, 2003).

Considering that periodontal regeneration requires a numbers of events including cementogenesis, osteogenesis and the insertion of new PDL into both cementum and bone the course of repair is not predictable. In this way, although the action of molecules from dental and periodontal tissues in these events is not totally clear, the experimental findings reflect the diversity of responses within the periodontium to specific factors, as well as highlighting the need to consider the complexity of factors involved in the design of regenerative therapies.

Dentin and bone molecules as regulators of bone cells activity

Several polypeptides sequestered in dentin matrix and also in bone have been reported as important factors in signaling chemotaxis, differentiation and activity of bone cells and, more generally, in the biologic processes involving tooth and surrounding tissues (Table 2). It is important to note that at sites of root and bone coupled resorption, both tissues may serve as sources of growth factors and other molecules, thus increasing the availability of these substances in this microenvironment.

Generally, the cellular events involved in resorption and healing of soft and hard dental structures include chemotaxis, proliferation, differentiation and activity. Chemotactic signals could include constituents of the dentin matrix or growth factors, such as TGF-β and PDGF, produced in their active form at the resorption sites (Tsahjian *et al*, 1985; Mochizuki *et al*, 1992). Subsequently the attracted cells present in this microenvironment would be affected by potent bone cell mitogens including IGF-I, IGF-II, PDGF and TGF-β (Tsahjian *et al*, 1985; Hock, Centrella and Canalis, 1988; Graves *et al*, 1989). In this way, dentin-derived growth factors may act in an autocrine and/or paracrine fashion regulating proliferation and differentiation of bone cells and playing important roles in the local regulation of coupled tooth-bone repair and resorption.

The complex network of cytokines that influence cell function includes TGF-β, a powerful stimulator of bone

cell growth, which has been clearly shown to stimulate bone formation *in vivo* (Noda and Camilliere, 1989). However, the *in vitro* effects of TGF-β appear to be highly dependent upon the cell source, the dose applied and the local environment, as seen in studies that demonstrated either stimulation (Locklin, Oreffo and Triffitt, 1999) or inhibition (Takata *et al*, 1998) of osteoblast proliferation. TGF-β also affects bone resorption by inhibiting osteoclast formation and activity (Chenu *et al*, 1988; Berghuis *et al*, 1994) or by stimulating bone resorption (Tsahjian *et al*, 1985).

Both acidic and basic FGFs are known to stimulate bone formation *in vivo* (Dunstan *et al*, 1999). However, *in vitro* studies have shown that while at low concentrations FGF-2 acts directly on mature osteoclasts to bring about moderate bone resorption, at high concentrations it acts on osteoblastic cells to induce cyclooxygenase-2, resulting in the synthesis of prostaglandins and a potent stimulation of bone resorption. Prostaglandins enhance the bone resorptive response to interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), which in turn are also potent stimuli for bone resorption and for prostaglandin release (Kawaguchi *et al*, 2000). There is evidence that FGFs can also affect bone resorption through direct and indirect mechanisms that promote angiogenesis and osteoclast recruitment, formation and differentiation (Hurley *et al*, 1998; Nakagawa *et al*, 1999; Collin-Osdoby *et al*, 2002).

The PDGF is a potent growth factor for various connective tissue cells being a powerful promoter of cell migration and proliferation. It has been shown that all isoforms of PDGF also promote DNA synthesis and chemotaxis in bone organ cultures (Graves *et al*, 1989). In a similar way to that observed for FGF, PDGF also stimulates bone resorption via the enhanced local production and release of prostaglandins (Tashjian *et al*, 1982).

The VEGF, an important angiogenic factor, was recently shown to increase osteoclast survival and activity (Nakagawa *et al*, 2000) in osteopetrotic mice, which have a severe osteoclast deficiency (Niida *et al*, 1999).

Two different IGFs, IGF-I and IGF-II, have been isolated from dentin (Finkelman *et al*, 1990). Both stimulate bone formation by inducing cellular proliferation, differentiation and the synthesis of type I collagen (Hock *et al*, 1988). IGFs have also been shown to have an effect on bone resorption by enhancing the generation of osteoclasts (Mochizuki *et al*, 1992; Hill, Reynolds and Meikle, 1995).

The BMPs 2, 3 and 7 have been identified in dentin during developmental stages of dentinogenesis (Thomadakis *et al*, 1999). Although *in vivo* the BMPs have the property of stimulating bone formation (King and Cochran, 2002), some investigations have reported BMP action on osteoclastogenesis and bone resorption (Hentunen *et al*, 1995; Kanatani *et al*, 1995; Koide *et al*, 1999).

The latent forms of MMPs incorporated into the organic dentin matrix may be involved in tissue remodeling under physiological and pathological conditions. However, the role of MMPs in osteoclast function is less clear. The most compelling evidence that these enzymes participate in the resorptive process comes from the demonstration that bone resorption is attenuated in mice carrying a mutation in the site on type I collagen that is targeted by neutral collagenases (Zhao *et al*, 1999). MMPs have been implicated in root degradation during tooth movement (Domon *et al*, 1999) and deciduous physiological resorption (Linsuwanont *et al*, 2002).

Noteworthy is the recent detection in odontoblasts, ameloblasts and pulp cell lines of factors that are constitutively expressed and which regulate osteoclastogenesis and bone resorption; these include: macrophage colony-stimulating factor, osteoprotegerin (OPG) and osteoclast differentiation factor/receptor-activator of NFκB ligand (ODF/RANKL) (Sakata *et al*, 1999; Rani and MacDougall, 2000). Furthermore, PDL cells express both RANKL and OPG mRNA, regulating osteoclastogenesis by opposing mechanisms of stimulation of resorptive activity by RANKL and inhibition by OPG (Kanzaki *et al*, 2001). These results corroborate the hypothesis that dentin constituents are involved in the process of resorption; however, the contribution of factors from dental cells to the osteoclastic activity in this pathological condition is unclear at present. OPG secreted by dental mesenchyme cells may play an important paracrine role as a decoy receptor, binding to RANKL on osteoblasts/stromal cells in the bone microenvironment surrounding teeth and thereby inhibiting osteoclast maturation. By contrast, in other conditions associated with inflammatory lesions, such as apical resorption, and during teeth shedding when matrix resorption activity is high these factors may be regulated differently so as to favor osteoclast formation.

Some investigators have examined the effects of dentin-bone matrix proteins on the activities of differentiated bone cells (Sato *et al*, 1992; Ingram *et al*, 1993; Zhou *et al*, 1995; Raynal, Delmas and Chenu, 1996; Razzouk *et al*, 2002) and on precursors of immature osteoclasts (Mundy *et al*, 1978; Malone *et al*, 1982; Glowacki *et al*, 1991; Chenu *et al*, 1994; Liggett *et al*, 1994). Mononuclear phagocytes, but not lymphocytes nor polymorphonuclear leukocytes, exhibit directional migration when placed in a gradient of conditioned medium collected from cultures of resorbing bone rudiments (Mundy *et al*, 1978). Similarly, Malone *et al* (1982) demonstrated that human peripheral monocytes, but not polymorphonuclear leukocytes, respond chemotactically to OC and α2HS glycoprotein as well as to collagen peptides.

Bone acid glycoprotein 75 (BAG 75), OPN and bone sialoprotein (BSP) are effective in inhibiting bone resorption (Sato *et al*, 1992). Likewise, Arg-Gly-Asp (RGD)-containing matrix proteins inhibit the resorption of bone by isolated rat osteoclasts. BAG 75, which is secreted by osteoblastic cells and enriched in areas surrounding newly calcified osteoid, may function *in vivo* to prevent the degradation by osteoclasts of newly forming bone until it is degraded by proteases.

There is increasing evidence that BSP might play a role in bone remodeling. BSP, along with OPN, are the only

non-collagenous bone matrix proteins known to be expressed by osteoclasts. BSP has stimulatory effects on proliferation, differentiation, and calcification in untransformed clonal MC3T3-E1 cells from newborn mouse calvariae with a preosteoblast phenotype (Zhou *et al*, 1995). In contrast to these results, it has been shown that BSP promotes resorption by stimulating cell attachment and osteoclast activity (Raynal *et al*, 1996; Razzouk *et al*, 2002). Although BSP stimulates bone resorption in a coculture system of bone marrow cells and osteoblastic cells, it causes a dose-dependent inhibition of the formation of osteoclast-like cells at equivalent concentrations in this culture model. The stimulatory effects of BSP on bone resorption were partially explained by an increase in osteoclast adhesion to bone via its RGD sequence, but the mechanism of action of BSP might also involve another region of the molecule. These observations support a role for BSP in bone remodeling but the biologic role of BSP in resorption is still unclear. Dentin sialoprotein, another sialic acid-rich protein, which lacks the RGD sequence, is not able to promote the attachment and spreading of gingival fibroblasts or dental papilla cells (Butler *et al*, 1992).

Osteopontin may facilitate the adhesion (or detachment) of osteoclasts at the bone surface, and may be a postresorptive signal for the recruitment of osteoblasts. Alternatively it may polarize and direct the mineralization of the formed osteoid, as it has been confirmed that osteoclasts are responsible for the deposition of OPN (Dodds *et al*, 1995). It has also been shown that OPN stimulates bone resorption (Razzouk *et al*, 2002).

Osteocalcin also stimulates *in vitro* osteoclast differentiation (Chenu *et al*, 1994; Liggett *et al*, 1994). Both *in vitro* and *in vivo* models suggest that OC is important for osteoclast recruitment (Glowacki *et al*, 1991; Chenu *et al*, 1994; Liggett *et al*, 1994). In culture OC is chemotactic for osteoclasts, implying that it is involved in bone remodeling (Malone *et al*, 1982). In support of this concept there are reports that OC stimulates osteoclast differentiation *in vitro* (Glowacki *et al*, 1991; Chenu *et al*, 1994; Liggett *et al*, 1994). The function of OC, as suggested by analysis of genetically engineered OC-deficient mice, is the inhibition of bone formation (Ducy *et al*, 1996). OC-deficient mice show an increase in bone formation without changes in osteoclast or osteoblast numbers. In a subsequent study Boskey *et al* (1998) demonstrated that OC also plays a role in bone crystal maturation, being required to stimulate bone mineral maturation. Furthermore, the OC receptor was recently shown to be expressed in human osteoblasts (Bodine and Komn, 1999).

Dentin as an inductor of the inflammatory and immune response

Dentin exposure *in vivo* is generally accompanied by osteoclasts colonization and resorption of the root surface. The course of resorption depends on a complex interaction of inflammatory and bone cells within the tooth and surrounding tissues (Ne *et al*, 1999). As root resorption is usually coupled to a local inflammatory process, it is probable that the inflammatory cells present at the resorption site come into contact with dentin molecules released into this microenvironment. Considering this hypothesis, the induction of inflammatory events by dentin may contribute to the maintenance of this process and, consequently, may affect the course of resorption, a phenomenon that has been stressed concerning bone resorption. We have examined the role of crude extracts of demineralized dentin as inductors of the inflammatory response. Dentin extracts trigger an *in vivo* leukocyte migration in a time and dose-dependent manner and the *in vitro* expression of inducible nitric oxide synthase, TNF-α and IL-1β by macrophages (Lara *et al*, 2003). Consistent with these observations dentin molecules, such as OPN and TGF-β, induce the recruitment and activation of leukocytes (Wahl *et al*, 1993a; Giachelli *et al*, 1998).

Osteopontin is a multifunctional cytokine and adhesion protein that exists both as an immobilized molecule in mineralized tissues and as a cytokine in body fluid. It is also known as early T-lymphocyte activator-1 and its interaction with macrophages can induce an inflammatory response (Denhardt and Noda, 1998). OPN appears to be involved in the maintenance and remodeling of tissue during inflammation, thus participating in wound healing, bone remodeling, and cell-mediated immunity (Denhardt and Noda, 1998; Denhardt *et al*, 2001). Intradermal injection of OPN leads to the infiltration of inflammatory cells, largely macrophages, as well as a small increase in polymorphonuclear cell numbers (Giachelli *et al*, 1998).

The TGF-β facilitates leukocyte adhesion to the vessel wall and to the extracellular matrix at the site of inflammation by increasing integrin expression. In addition, administration of anti-TGF-β antibodies blocked leukocyte recruitment and activation and also tissue destruction at sites of chronic destructive inflammation (Wahl *et al*, 1993a).

Odontoblasts exhibit constitutive expression of the pro-inflammatory mediator IL-8, which has been shown to be chemotactic for neutrophils, T lymphocytes and basophils, and also to stimulate neutrophil degranulation and oxidative burst activity, as well as to stimulate histamine release from mast cells. As this cytokine may be sequestered in dentin, its release might contribute to perpetuate and/or exacerbate the inflammatory response in pulp and periapex (Levin *et al*, 1999).

Therefore, the existence of modulators and down-regulators in this system should be considered. This concept is supported by data demonstrating that dentin sialophosphoprotein (DSPP), OPN, BSP and dentin matrix protein-1, collectively named the SIBLINGs (Small Integrin-Binding Ligand, N-linked Glycoprotein) family on the basis of shared biochemical and genetic features, can interfere with the lytic pathway of complement quenching inflammatory responses involving alternative complement pathways (Jain *et al*, 2002).

Besides non-specific inflammatory responses, more specific immune responses have been elicited by dentin extracellular matrix proteins. Although the hypothesis that the immune system is linked to root resorption has been abandoned in the last 10 years, the ability of

mineralized dental tissues to elicit an immune response remains a matter of controversy. It has been observed that the subperiosteal implantation of allogenous decalcified sterilized dentin elicits a measurable alloimmune response (Schwartz, 1983). In previous studies concerning immune reactions after allotransplantation in animals, a similar humoral response was demonstrated after transplantation of calcified allogenic dental hard tissues (Mincer and Jennings, 1970; Riviere and Hansen, 1973). Bang (1972), using the skin rejection test observed a strong inflammatory reaction with many eosinophils and multinucleated giant cells, such as foreign body giant cells, 5 weeks after implantation of demineralized allogenic dentin. Conversely, autogenous dentin caused no inflammatory reaction.

Ng, King and Courts (1990) used dog and mouse models to show that after inducing incisor root resorption there is a decrease in dentin serum antibody titers along with root resorption. From this observation these authors suggested that a resorbing root surface may act as an antigen sink that absorbs antibody from the serum, or perhaps a suppressor T-cell population may have been activated to down-regulate the production of antibody. Subsequently, in order to confirm these observations Wheeler and Stroup (1993) investigated whether elevated antibody levels obtained after hyperimmunization with dentin would affect tooth root resorption. These authors found that the dentin immunization procedure can prevent traumatic root resorption and that increased dentin antibody titers may protect mice from this process. This supports the hypothesis that dental constituents can be active during root resorption by inducing specific or non-specific inflammatory responses.

Figure 1 depicts a hypothetical model for the action of dentin molecules at sites of repair and resorption. We suggested that dentin molecules released as a result of matrix dissolution and trafficking pathways in osteoclasts during the resorption process (Nesbitt and Horton, 1997) may be able to act as chemotactic and activator signals for inflammatory (Malone *et al*, 1982; Wahl *et al*, 1993b; Denhardt and Noda, 1998; Giachelli *et al*, 1998; Denhardt *et al*, 2001) and bone resorbing cells (Chenu *et al*, 1988; Mochizuki *et al*, 1992; Hurley *et al*, 1998; Koide *et al*, 1999; Nakagawa *et al*, 1999). Dentin molecules stimulate macrophages to produce the inflammatory mediators IL-1β, TNF-α and nitric oxide (NO) (Lara *et al*, 2003) and they have been implicated as up-regulators of bone and root resorption. Dentin also contains molecules that induce repair by stimulating the differentiation and activity of osteoblasts (Zhou *et al*, 1995; Yamaguchi, Komori and Suda, 2000) and chemotaxis and proliferation of PDL cells (Terranova *et al*, 1989; Matsuda *et al*, 1992). This scenario supports the potential dual regulation of tooth-periodontal repair and resorption; however, conditions that shift the balance in favor of destruction or rebuilding have not been determined. Further studies will be necessary to elucidate what molecules are in fact released at sites of dentin matrix dissolution and whether their neutralization has an influence on inflammatory and bone cell activity and, therefore, on tooth/periodontal pathological processes.

Concluding remarks

In this review, we present data from the literature concerning the ability of dentin to induce cellular

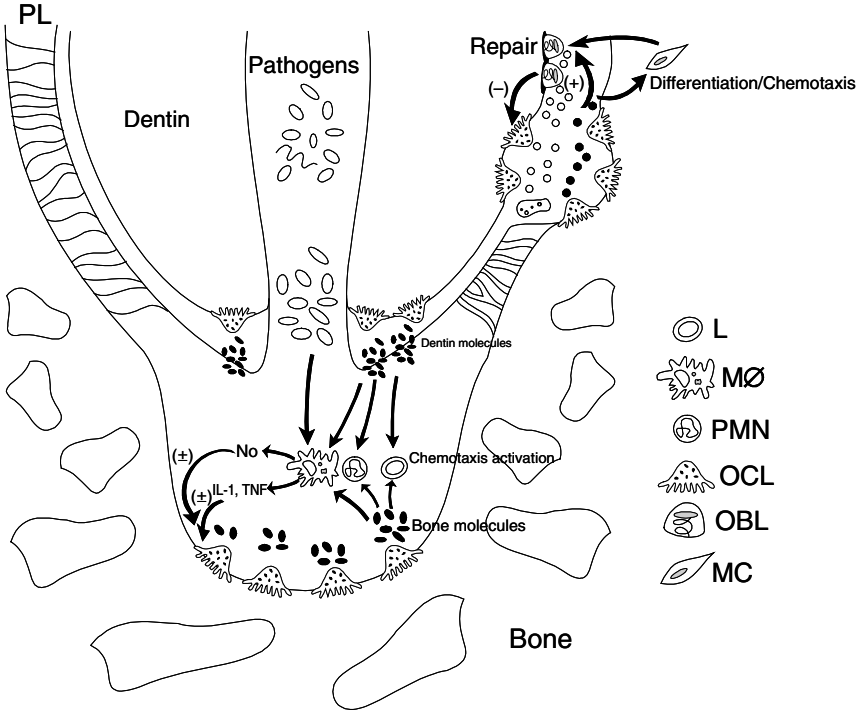

Figure 1 Hypothetical model for interactions of dentin and bone molecules and the cellular mechanisms of tooth resorption and repair. The different polypeptides entrapped in dentin, when locally released may act as signaling molecules for mesenchymal cells, osteoblasts, osteoclasts and inflammatory cells. Besides the dual effect of dentin molecules stimulating the repair following resorption and/or contributing to the maintenance of the resorption process, the cellular mechanisms are dependent on local factors such as bacteria, extent of damage to dentin and adjacent bone and the intensity of associated inflammatory processes. Inflammatory cells may be recruited and activated to release inflammatory mediators such as NO, IL-1 and TNF, which in turn stimulate osteoclastic activity. In a single root different stages in these processes may take place simultaneously. PL, periodontal ligament; L, lymphocytes; MØ, macrophages; PMN, polymorphonuclear leukocytes; OCL, osteoclasts; OBL, osteoblasts; MC, mesenchymal cells; NO, nitric oxide; IL-1, interleukin-1; TNF, tumor necrosis factor

responses. These studies reveal dentin as a source of signaling molecules able to elicit responses that could favor the building or destruction of dental and periodontal tissues. Considering the antagonistic activities attributed to different dentin constituents and that much of this information has come from *in vitro* experiments, it is not possible to establish the actual participation in the progression of dental diseases of the complex mixture of molecules from hard and soft dental and periodontal tissues. Thus, a number of questions need to be posed. First, does immobilization in a mineralized matrix stabilize the protein and preserve its activity over the long term? If stabilized, can a functional molecule be released upon demineralization caused either experimentally or pathologically, as in dental caries, periodontal disease and tooth resorption? Is the availability of biologically active molecules influenced by local conditions? Finally, are sufficient amounts of active proteins released to make a significant contribution to the regulation of resorption or the induction of repair during pathological exposure?

It is clear that the processes that occur at the interface of soft and hard dental tissues are very tightly regulated, depending on the sequential expression of a number of factors that interact to ensure orderly progression and to determine whether the balance will be in favor of building or destruction. There is compelling evidence that the release of dentin-derived bioactive factors as a result of exposure or destruction of dentin matrix can contribute to vigorous chemotaxis and aggregation of inflammatory cells at tooth-periodontal sites and, consequently, influence the maintenance and or resolution of this process. We reconstructed the scenario of healing and resorption of dental tissues taking into account recent advances in the understanding of dentin molecular composition and cellular interactions. Bearing in mind that the understanding of these complex interactions is only at the beginning, and that much of the information on this cascade has come from *in vitro* studies, we presented evidence that dentin factors orchestrate at least one part of this scenario. Some of these biologic mediators show potential for clinical use in areas such as periodontology and restorative dentistry providing new approaches to the treatment of dental diseases. Finally, the molecular components of dentin represent prime targets for future structure-function studies *in vivo* and *in vitro*.

Acknowledgements

The authors are indebted to Dr W.T. Butler and Dr F.Q. Cunha for their valuable suggestions in reviewing this manuscript. T.A.S. was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Bang G (1972). Induction of heterotopic bone formation by demineralized dentin in guinea pigs: antigenicity of the dentin matrix. *J Oral Pathol* **1:** 172–185.

Bang G, Urist MR (1967). Bone induction in excavation chambers in matrix of decalcified dentin. *Arch Surg* **94:** 781–789.

Berghuis HM, Dieudonné SC, Goei W *et al* (1994). Effects of TGF-β2 on mineral resorption in cultured embryonic mouse long bones: ^{45}Ca release and osteoclast differentiation and migration. *Eur J Orthod* **16:** 130–137.

Bessho K, Tagawa T, Murata M (1990). Purification of rabbit bone morphogenetic protein derived from bone, dentin, and wound tissue after tooth extraction. *J Oral Maxillofac Surg* **48:** 162–169.

Bessho K, Tanaka N, Matsumoto J *et al* (1991). Human dentin-matrix-derived bone morphogenetic protein. *J Dent Res* **70:** 171–175.

Bodine PVN, Komn BS (1999). Evidence that conditionally immortalized human osteoblasts express an osteocalcin receptor. *Bone* **25:** 535–543.

Booth V, Young S, Cruchley A *et al* (1998). Vascular endothelial growth factor in human periodontal disease. *J Periodontal Res* **33:** 491–499.

Boskey AL, Gadaleta S, Gundberg C *et al* (1998). Fourier transform infrared microspectroscopic analysis of bones of osteocalcin-deficient mice provides insight into the function of osteocalcin. *Bone* **23:** 187–196.

Butler WT (1998). Dentin matrix proteins. *Eur J Oral Sci* **106** (Suppl. 1)**:** 204–210.

Butler WT, Bhown M, Brunn JC *et al* (1992). Isolation, characterization and immunolocalization of a 53-kDal dentin sialoprotein (DSP). *Matrix* **12:** 343–351.

Cassidy N, Fahey M, Prime SS *et al* (1997). Comparative analysis of transforming growth factor β isoforms 1–3 in human and rabbit dentin matrices. *Arch Oral Biol* **42:** 219–223.

Chenu C, Pfeilschifter J, Mundy GR *et al* (1988). Transforming growth factor β inhibits formation of osteoclast-like cells in long-term human marrow cultures. *Proc Natl Acad Sci USA* **85:** 5683–5687.

Chenu C, Colucci S, Grano M *et al* (1994). Osteocalcin induces chemotaxis, secretion of matrix proteins, and calcium-mediated intracellular signaling in human osteoclast-like cells. *J Cell Biol* **127:** 1149–1158.

Collin-Osdoby P, Rothe L, Bekker S *et al* (2002). Basic fibroblast growth factor stimulates osteoclast recruitment, development, and bone pit resorption in association with angiogenesis *in vivo* on the chick chorioallantoic membrane and activates isolated avian osteoclast resorption *in vitro*. *J Bone Miner Res* **17:** 1859–1871.

Denhardt DT, Noda M (1998). Osteopontin expression and function role in bone remodeling. *J Cell Biochem* **30–31** (Suppl.)**:**92–102.

Denhardt DT, Noda M, O'Reagan AW *et al* (2001). Osteopontin as means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. *J Clin Invest* **107:** 1055–1061.

Dodds RA, Connor JR, James IE *et al* (1995). Human osteoclasts, not osteoblasts, deposit osteopontin onto resorption surfaces: an *in vitro* and *ex vivo* study of remodeling bone. *J Bone Miner Res* **10:** 1666–1680.

Domon S, Shimokawa H, Matsumoto Y *et al* (1999). In situ hybridization for matrix metalloproteinase-1 and cathepsin K in rat root-resorbing tissue induced by tooth movement. *Arch Oral Biol* **44:** 907–915.

Ducy P, Desbois C, Boyce B *et al* (1996). Increased bone formation in osteocalcin-deficient mice. *Nature* **382:** 448–452.

Dunstan CR, Boyce R, Boyce BF *et al* (1999). Systemic administration of acidic fibroblast growth factor (FGF-1) prevents bone loss and increases new bone formation in ovariectomized rats. *J Bone Miner Res* **14:** 953–959.

Ejeil AL, Igondjo-Tchen S, Ghomrasseni S *et al* (2003). Expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in healthy and diseased human gingiva. *J Periodontol* **74:** 188–195.

Finkelman RD (1992). Growth factors in bones and teeth. *J Calif Dent Assoc* **20:** 23–29.

Finkelman RD, Mohan S, Jennings JC *et al* (1990). Quantification of growth factors IGF-I, SGF/IGFII and TGF-beta in human dentin. *J Bone Miner Res* **5:** 717–723.

Fujisawa R, Butler WT, Brunn JC *et al* (1993). Differences in composition of cell-attachment sialoproteins between dentin and bone. *J Dent Res* **72:** 1222–1226.

Gao Y, Yang L, Fang YR *et al* (1995). The inductive effect of bone morphogenetic protein (BMP) on human periodontal fibroblast-like cells *in vitro*. *J Osaka Dent Univ* **29:** 9–17.

Giachelli CM, Lombardi D, Johnson RJ *et al* (1998). Evidence for a role of osteopontin in macrophage infiltration in response to pathological stimuli *in vivo*. *Am J Pathol* **152:** 353–358.

Glowacki J, Rey C, Glimcher MJ *et al* (1991). A role for osteocalcin in osteoclast differentiation. *J Cell Biochem* **45:** 292–302.

Graves DT, Valentin-Opran A, Delgado R *et al* (1989). The potential role of platelet-derived growth factors as an autocrine and paracrine factor for human bone cells. *Connect Tissue Res* **23:** 209–218.

Hasegawa N, Kawaguchi H, Ogawa T *et al* (2003). Immunohistochemical characteristics of epithelial cell rests of Malassez during cementum repair. *J Periodontal Res* **38:** 51–56.

Hentunen TA, Lakkakorpi PT, Tuukknen J *et al* (1995). Effects of recombinant human osteogenic protein-1 on the differentiation of osteoblast-like cells and bone resorption. *Biochem Biophys Res Commun* **209:** 433–443.

Heras SM-D, Valenzuela A, Overall CM (2000). The matrix metalloproteinase gelatinase A in human dentine. *Arch Oral Biol* **45:** 757–765.

Hill PA, Reynolds JJ, Meikle MC (1995). Osteoblasts mediate insulin-like growth factor-I and -II stimulation of osteoclast formation and function. *Endocrinology* **136:** 124–131.

Hock JM, Centrella M, Canalis E (1988). Insulin-like growth factor I (IGF-I) has independent effects on bone matrix formation and cell replication. *Endocrinology* **122:** 254–260.

Hoffmann A, Weich HA, Gross G *et al* (2001). Perspectives in the biological function, the technical and therapeutic application of bone morphogenetic proteins. *Appl Microbiol Biotechnol* **57:** 294–308.

Huggins C, Wiseman S, Reddi AH (1970). Transformation of fibroblasts by allogenic and xenogenic transplants of demineralized tooth and bone. *J Exp Med* **132:** 1250–1258.

Hurley MM, Lee SK, Raisz LG *et al* (1998). Basic fibroblast growth factor induces osteoclast formation in murine bone marrow cultures. *Bone* **22:** 309–316.

Ingram RT, Clarke BL, Fisher LW *et al* (1993). Distribution of noncollagenous proteins in matrix of adult human bone: evidence of anatomic and functional heterogeneity. *J Bone Miner Res* **8:** 1019–1029.

Inoue T, Deporter DA, Melcher AH (1986). Induction of chondrogenesis in muscle, skin, bone marrow and periodontal ligament by demineralized dentin and bone matrix *in vivo* and *in vitro*. *J Dent Res* **65:** 12–22.

Inoue T, Chen SH, Shimono M (1990). Induction of cartilage and bone formation by cells from explants of various oral tissues *in vitro*. *Bull Tokyo Dent Coll* **31:** 295–300.

Ivanovski S, Haase HR, Bartold PM (2001). Expression of bone matrix protein mRNAs by primary and cloned cultures of the regenerative phenotype of human periodontal fibroblasts. *J Dent Res* **80:** 1665–1671.

Jain A, Karadag A, Fohr B *et al* (2002). Three SIBLINGs (small integrin-binding ligand, N-linked glycoproteins) enhance factor H's cofactor activity enabling MCP-like cellular evasion of complement-mediated attack. *J Biol Chem* **277:** 13700–13708.

Johnson RB, Serio FG, Dai X (1999). Vascular endothelial growth factors and progression of periodontal diseases. *J Periodontol* **70:** 848–852.

Kanatani M, Sugimoto T, Kaji H *et al* (1995). Stimulatory effect of bone morphogenetic protein-2 on osteoclast-like cell formation and bone resorbing activity. *J Bone Miner Res* **10:** 1681–1690.

Kanzaki H, Chiba M, Shimizu Y *et al* (2001). Dual regulation of osteoclast differentiation by periodontal ligament cells through RANKL stimulation and OPG inhibition. *J Dent Res* **80:** 887–891.

Kawaguchi H, Chikazu D, Nakamura K *et al* (2000). Direct and indirect actions of fibroblast growth factor 2 on osteoclastic bone resorption in cultures. *J Bone Miner Res* **15:** 466–473.

Kawai T, Urist MR (1989). Bovine tooth-derived bone morphogenetic protein. *J Dent Res* **68:** 1069–1074.

King GN, Cochran DL (2002). Factors that modulate the effects of bone morphogenetic protein-induced periodontal regeneration: a critical review. *J Periodontol* **73:** 925–936.

King GN, Hughes FJ (2001). Bone morphogenetic protein-2 stimulates cell recruitment and cementogenesis during early wound healing. *J Clin Periodontol* **28:** 465–475.

Koide M, Murase Y, Yamato K *et al* (1999). Bone morphogenetic protein-2 enhances osteoclast formation mediated by interleukin-1α through upregulation of osteoclast differentiation factor and cyclooxygenase-2. *Biochem Biophys Res Commun* **259:** 97–102.

Lara VS, Figueiredo F, Silva TA *et al* (2003). Dentin induced *in vivo* inflammatory response and *in vitro* activation of murine macrophages. *J Dent Res* **82:** 460–465.

Levin LG, Rudd A, Bletsa A *et al* (1999). Expression of IL-8 by cells of the odontoblast layer *in vitro*. *Eur J Oral Sci* **107:** 131–137.

Liggett WH, Lian JB, Greenberger JS *et al* (1994). Osteocalcin promotes differentiation of osteoclast progenitors from murine long-term bone marrow cultures. *J Cell Biochem* **55:** 190–199.

Linde, A (1989). Dentin matrix proteins: composition and possible functions in calcification. *Anat Rec* **224:** 154–166.

Linsuwanont B, Takagi Y, Ohya K *et al* (2002). Expression of matrix metalloproteinase-9 mRNA and protein during deciduous tooth resorption in bovine odontoclasts. *Bone* **31:** 472–478.

Locklin RM, Oreffo ROC, Triffitt JT (1999). Effects of TGF and bFGF on the differentiation of human bone marrow stromal fibroblasts. *Cell Biol Int* **23:** 185–194.

Malone JD, Teitelbaum SL, Griffin GL *et al* (1982). Recruitment of osteoclast precursors by purified bone matrix constituents. *J Cell Biol* **92:** 227–230.

Matsuda N, Lin WL, Kumar NM *et al* (1992). Mitogenic, chemotactic, and synthetic responses of rat periodontal ligament fibroblastic cells to polypeptide growth factors *in vitro*. *J Periodontol* **63:** 515–525.

Matsuda N, Kumar NM, Ramakrishnan PR *et al* (1993). Evidence for up-regulation of epidermal growth factor receptors on rat periodontal ligament fibroblast cells asso-

ciated with stabilization of phenotype *in vitro*. *Arch Oral Biol* **38:** 559–569.

Miki Y, Narayanan AS, Page RC (1987). Mitogenic activity of cementum components to gingival fibroblasts. *J Dent Res* **66:** 1399–1403.

Mincer HH, Jennings BR (1970). Morphologic and immunologic studies of subcutaneous incisor allografts in mice. *J Dent Res* **49:** 381–388.

Mochizuki H, Hakeda Y, Wakatsuki N *et al* (1992). Insulin-like growth factor-I supports formation and activation of osteoclasts. *Endocrinology* **131:** 1075–1080.

Mumford JH, Carnes DL, Cochran DL *et al* (2001). The effects of platelet-derived growth factor-BB on periodontal cells in an *in vitro* wound model. *J Periodontol* **72:** 331–340.

Mundy GR, Varani J, Orr W *et al* (1978). Resorbing bone is chemotactic for monocytes. *Nature* **275:** 132–135.

Murakami S, Takayama S, Ikezawa K *et al* (1999). Regeneration of periodontal tissues by basic fibroblast growth factor. *J Periodontal Res* **34:** 425–430.

Nakagawa N, Yasuda H, Yano K *et al* (1999). Basic fibroblast growth factor induces osteoclast formation by reciprocally regulating the production of osteoclast differentiation factor and osteoclastogenesis inhibitory factor in mouse osteoclastic cells. *Biochem Biophys Res Commun* **265:** 158–163.

Nakagawa M, Kaneda T, Arakawa T *et al* (2000). Vascular endothelial growth factor (VEGF) directly enhances osteoclastic bone resorption and survival of mature osteoclasts. *FEBS Lett* **473:** 161–164.

Ne RF, Whiterspoon DE, Gutmann JL (1999). Tooth resorption. *Quintessence Int* **30:** 9–25.

Nebgen DR, Inoue H, Sabsay B *et al* (1999). Identification of the chondrogenic-inducing activity from bovine dentin (bCIA) as a low-molecular-mass amelogenin polypeptide. *J Dent Res* **78:** 1484–1494.

Nesbitt SA, Horton MA (1997). Trafficking of matrix collagens through bone-resorbing osteoclasts. *Science* **276:** 266–269.

Ng KT, King GJ, Courts FJ (1990). Humoral immune response to active root resorption with a murine model. *Am J Orthod Dentofacial Orthop* **98:** 456–462.

Niida S, Kaku M, Amano H *et al* (1999). Vascular endothelial growth factor can substitute for macrophage colony-stimulating factor in the support of osteoclastic bone resorption. *J Exp Med* **190:** 293–298.

Nishimura K, Hayashi M, Matsuda K *et al* (1989). The chemoattractive potency of periodontal ligament, cementum and dentin for human gingival fibroblasts. *J Periodontal Res* **24:** 146–148.

Noda M, Camilliere JJ (1989). *In vivo* stimulation of bone formation by transforming growth factor β. *Endocrinology* **124:** 2991–2994.

Ogata Y, Niisato N, Moriwaki K *et al* (1997). Cementum, root dentin and bone extracts stimulate chemotactic behavior in cells from periodontal tissue. *Comp Biochem Physiol* **116:** 359–365.

Palosaari H, Wahlgren J, Larmas M *et al* (2000). Expression of MMP-8 in human odontoblasts and dental pulp cells is down-regulated by TGF-β-1. *J Dent Res* **79:** 77–84.

Parkar MH, Kuru L, Giouzeli M *et al* (2001). Expression of growth-factor receptors in normal and regenerating human periodontal cells. *Arch Oral Biol* **46:** 275–284.

Qin C, Brunn JC, Cadena E *et al* (2002). The expression of dentin sialophosphoprotein gene in bone. *J Dent Res* **81:** 392–394.

Rabinowitz T, Syftestad GT, Caplan AI (1990). Chondrogenic stimulation of embryonic chick limb mesenchymal cells by factors in bovine and human dentin extracts. *Arch Oral Biol* **35:** 49–54.

Rani CSS, MacDougall M (2000). Dental cells express factors that regulate bone resorption. *Mol Cell Biol Res Commun* **3:** 145–152.

Raynal C, Delmas PD, Chenu C (1996). Bone sialoprotein stimulates *in vitro* bone resorption. *Endocrinology* **137:** 2347–2354.

Razzouk S, Brunn JC, Qin C *et al* (2002). Osteopontin posttranslational modifications, possibly phosphorylation, are required for *in vitro* bone resorption but not osteoclast adhesion. *Bone* **30:** 40–47.

Ripamonti U, Heliotis M, Rueger DC *et al* (1996). Induction of cementogenesis by recombinant human osteogenic protein-1 (hop-1/bmp-7) in the baboon (*Papio ursinus*). *Arch Oral Biol* **41:** 121–126.

Riviere GR, Hansen RW (1973). Alloantibodies induced in histoincompatible rats by single pulp and tooth grafts. *J Dent Res* **52:** 1186–1188.

Roberts-Clark DJ, Smith AJ (2000). Angiogenic growth factors in human dentine matrix. *Arch Oral Biol* **45:** 1013–1016.

Sakata M, Shiba H, Komatsuzawa H *et al* (1999). Expression of osteoprotegerin (osteoclastogenesis inhibitory factor) in cultures of human dental mesenchymal cells and epithelial cells. *J Bone Miner Res* **14:** 1486–1492.

Sato M, Grasser W, Harm S *et al* (1992). Bone acid glycoprotein 75 inhibits resorption activity of isolated rat and chicken osteoclasts. *FASEB J* **6:** 2966–2976.

Saygin NE, Tokiyasu Y, Giannobile WV *et al* (2000). Growth factors regulate expression of mineral associated genes in cementoblasts. *J Periodontol* **71:** 1591–1600.

Scheven BAA, Marshall D, Aspden RM (2002). *In vitro* behaviour of human osteoblasts on dentin and bone. *Cell Biol Int* **26:** 337–346.

Schwartz, O (1983). Cell-mediated and humoral alloimmune reactions after subperiosteal implantation of allogenic demineralized dentin in humans. *Int J Oral Surg* **12:** 95–105.

Schwartz Z, Lohmann CH, Wieland M *et al* (2000). Osteoblast proliferation and differentiation on dentin slices are modulated by pretreatment of the surface with tetracycline or osteoclasts. *J Periodontol* **71:** 586–597.

Smith AJ, Lesot H (2001). Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? *Crit Rev Oral Biol Med* **12:** 425–437.

Somerman MJ, Archer SY, Hassell TM *et al* (1987a). Enhancement by extracts of mineralized tissues of protein production by human gingival fibroblasts *in vitro*. *Arch Oral Biol* **32:** 879–883.

Somerman MJ, Perez-Mera M, Merkhofer RM *et al* (1987b). *In vitro* evaluation of extracts of mineralized tissues for their application in attachment of fibrous tissue. *J Periodontol* **58:** 349–351.

Somerman MJ, Foster RA, Imm GM *et al* (1989). Periodontal ligament cells and gingival fibroblasts respond differently to attachment factors *in vitro*. *J Periodontol* **60:** 73–77.

Steinsvoll S, Halstensen TS, Schenck K (1999). Extensive expression of TGF-beta1 in chronically-inflamed periodontal tissue. *J Clin Periodontol* **26:** 366–373.

Takata T, D'Errico JA, Atkins KB *et al* (1998). Protein extracts of dentin affect proliferation and differentiation of osteoprogenitor cells *in vitro*. *J Periodontol* **69:** 1247–1255.

Takayama S, Murakami S, Nozaki T *et al* (1998). Expression of receptors for basic fibroblast growth factor on human periodontal ligament cells. *J Periodontal Res* **33:** 315–322.

Tashjian AH, Jr, Hohmann EL, Antoniades HN *et al* (1982). Platelet-derived growth factor stimulates bone resorption via a prostaglandin-mediated mechanism. *Endocrinology* **111:** 118–124.

Tenorio D, Foyle DM, Hughes FJ (1997). The modulatory role of cementum matrix on osteoblastic cells *in vitro*. *J Periodontal Res* **32:** 362–374.

Terranova VP, Odziemiec C, Tweden KS *et al* (1989). Repopulation of dentin surfaces by periodontal ligament cells and endothelial cells: effect of basic fibroblast growth factor. *J Periodontol* **60:** 293–301.

Thomadakis G, Ramoshebi LN, Crooks J *et al* (1999). Immunolocalization of bone morphogenetic protein-2 and -3 and osteogenic protein-1 during murine tooth root morphogenesis and in other craniofacial structures. *Eur J Oral Sci* **107:** 368–377.

Tjäderhane L, Salo T, Larjava H *et al* (1998). A novel organ culture method to study the function of the human odontoblasts *in vitro*: gelatinase expression by odontoblasts is differentially regulated by TGF-β-1. *J Dent Res* **77:** 1488–1498.

Tompkins K, Veis A (2001). Amelogenin gene splice product [A-4] binds to embryonic fibroblasts *in vitro*. *J Dent Res* **80:** 242 (Abstract).

Tsahjian AH, Levine L (1978). Epidermal growth factor stimulated prostaglandin production and bone resorption in cultured mouse calvaria. *Biochem Biophys Res Commun* **85:** 966–975.

Tsahjian AH, Voelkel EF, Lazzaro M *et al* (1985). Alpha and beta transforming growth factors stimulate prostaglandin production and bone resorption in cultured mouse calvaria. *Proc Natl Acad Sci* **82:** 4535–4538.

Urist MR, Strates BS (1971). Bone morphogenetic protein. *J Dent Res* **50:** 1392–1406.

Veis A, Sires B, Clohisy J (1989). A search for the osteogenic factor in dentin. Rat incisor dentin contains a factor stimulating rat muscle cells *in vitro* to incorporate sulfate into an altered proteoglycan. *Connect Tissue Res* **23:** 137–144.

Veis A, Sires B, Clohisy J *et al* (1990). Rat incisor dentine contains a factor which alters the phenotypic expression and stimulates chondrogenesis in fibroblast-like cells *in vitro*. *Biomaterials* **11:** 35–37.

Veis A, Tompkins K, Alvares K *et al* (2000). Specific amelogenin gene splice products have signaling effects on cells in culture and in implants *in vivo*. *J Biol Chem* **275:** 41263–41272.

Wahl SM, Allen JB, Costa GL *et al* (1993a). Reversal of acute and chronic synovial inflammation by anti-transforming growth factor beta. *J Exp Med* **177:** 225–230.

Wahl SM, Costa GL, Mizel DE *et al* (1993b). Role of transforming growth beta in pathophysiology of chronic inflammation. *J Periodontol* **64:** 450–455.

Wheeler TT, Stroup SE (1993). Traumatic root resorption in dentine-immunized mice. *Am J Orthod Dentofacial Orthop* **103:** 352–357.

Yamaguchi A, Komori T, Suda T (2000). Regulation of osteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs and cbfa1. *Endocr Rev* **21:** 393–411.

Yoemans JD, Urist MR (1967). Bone induction by decalcified dentin implanted into oral osseous and muscle tissues. *Arch Oral Biol* **12:** 999–1008.

Zhao WG, Byrne MH, Boyce BF *et al* (1999). Bone resorption induced by parathyroid hormone is strikingly diminished by parathyroid hormone in collagenase resistant mutant mice. *J Clin Invest* **103:** 517–524.

Zhao M, Berry JE, Somerman MJ (2003). Bone morphogenetic protein-2 inhibits differentiation and mineralization of cementoblasts *in vitro*. *J Dent Res* **82:** 23–27.

Zhou HY, Takita H, Fujisawa R *et al* (1995). Stimulation by bone sialoprotein of calcification in osteoblast-like MC3T3-E1 cells. *Calcif Tissue Int* **56:** 403–407.