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Scientific Section

Proteoglycans and Orthodontic Tooth Movement

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Abstract. *Proteoglycans represent an important and diverse family of extracellular matrix components within the connective tissues of the periodontium. This review focuses on the function and metabolism of the various proteoglycans in periodontal tissues, such as alveolar bone and periodontal ligament, and considers their potential fate in response to an orthodontic force. Such considerations provide an important background in evaluating the potential for proteoglycan metabolites, alongside other connective tissue metabolites, as biomarkers for assessing the deep-seated metabolic changes and as a diagnostic tool in monitoring orthodontic tooth movement.*

Index words: Proteoglycan, Glycosaminoglycan, Gingival crevicular fluid, orthodontic tooth movement.

Introduction

The value of the gingival crevicular fluid (GCF) in the assessment of the biological state of the deeper-seated tissues of the periodontium lends itself as a source of the biomarkers of specific clinical situations. This is of particular merit in monitoring the efficiency and outcomes of orthodontic treatment, primarily the response of alveolar bone to forced movement.

The pioneer studies by Embery, Last and Waddington during the past decade have indicated that the chondroitin sulphate proteoglycan, decorin, is a primary biomarker of alveolar bone. This knowledge has been deduced from a variety of clinical situations including advanced periodontal disease, osseo-integration, trauma from occlusion and orthodontic movement in parallel with sophisticated biochemical analysis (Waddington *et al*., 1994).

This review attempts to focus current knowledge in this area in an assessment of the state of the periodontal tissues in relation to orthodontic treatment. The limitations on clinical parameters for assessing metabolic changes in such tissues under the influence of tooth movement make the involvement of biochemical markers, particularly of bone, periodontal ligament and cementum, an exciting possibility with much potential for future diagnosis. This review directs attention of our current state of knowledge in this area and deals primarily with the non-collagenous components of the extracellular matrix of the periodontium with particular emphasis on the proteoglycan family.

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Components of the extracellular matrix

The extracellular matrix provides important functions within the connective tissues of the periodontium in maintaining structural integrity and regulation of cellular activity and function. The principal elements may be considered as a collagenous fibrous network providing structural support embedded in and interacting with a non-collagenous matrix consisting of proteoglycans and various glycoproteins. Within connective tissues proteoglycans represent an important and diverse family of glycoconjugates, comprised of a protein core to which one or more glycosaminoglycan chains are covalently attached. Both the protein and glycosaminoglycan moieties are important in considering their functional aspects within the extracellular matrix. The glycosaminoglycan chains are highly anionic linear chains consisting of a disaccharide repeating unit of a hexouronic acid and an *n*-acetyl hexosamine, the chemical detail of which are shown in Figure 1. Seven glycosaminoglycan species exist and, with the exception of hyaluronan, are invariably sulphated. Hyaluronan forms a further exception in that it is the only glycosaminoglycan in connective tissues found non-covalently attached to a protein core.

In recent years the nature, structure and biological activities of the protein core has provided a means of classifying the various proteoglycan families. The predominant families, together with named examples and details relating to the structure of proteoglycan species present within periodontal tissues are shown in Figure 2. For further detail of the structure function relationship of the various proteoglycan families the reader is referred to several recent reviews (Iozzo, 1998; Bartold and Narayanan, 1998).

Within the periodontal tissues perhaps one of the most important proteoglycan families are the SLRP. These macromolecules contain a protein core of 45–55kDa, which contain 7–24 leucine-rich repeat patterns (Figure 2). Bio-

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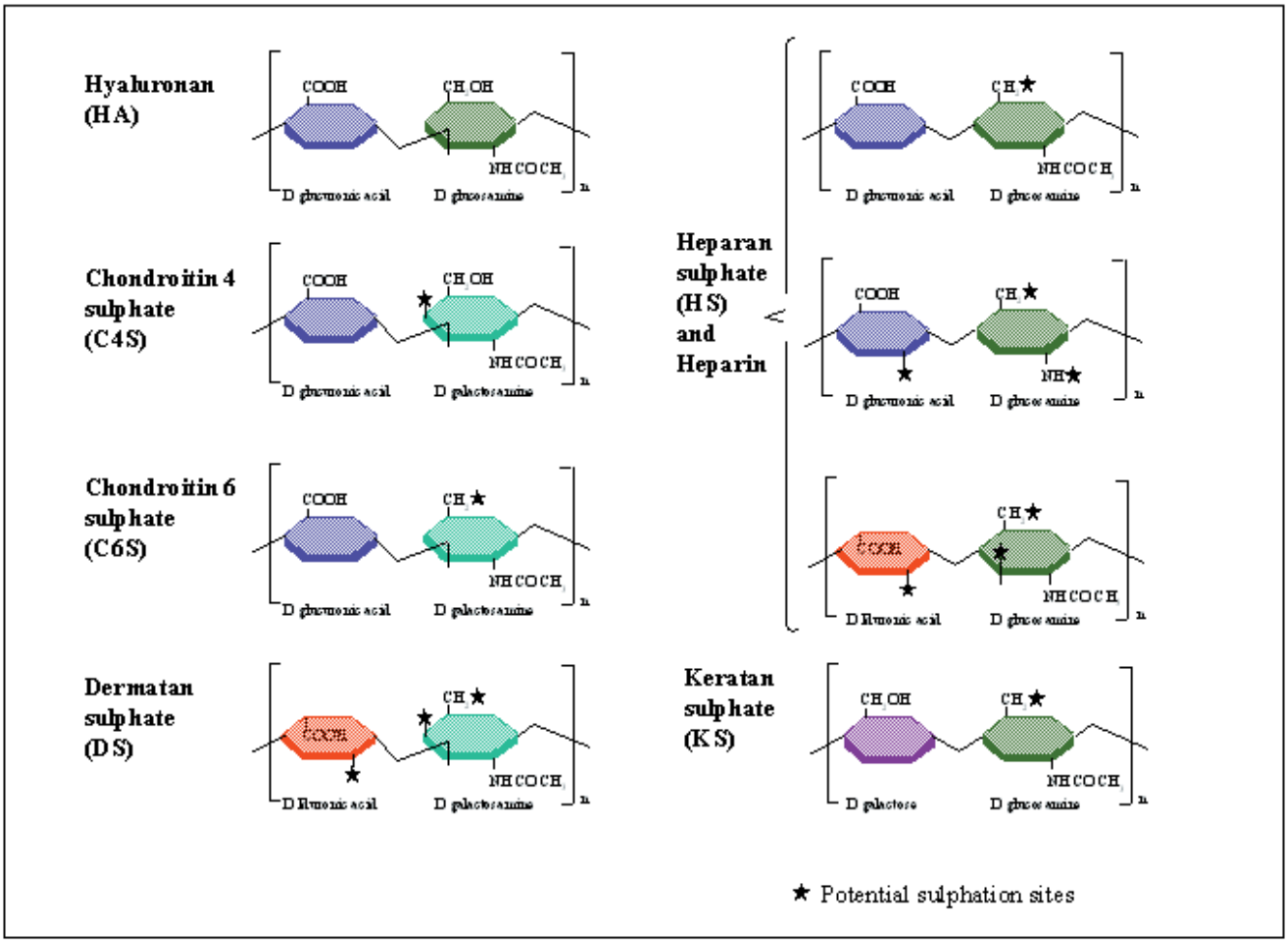


FIG. 1 The classification and repeat disaccharide units of glycosaminoglycans.

chemical and immunological analysis of mineralized connective tissues of alveolar bone and cementum have demonstrated that decorin and biglycan, carrying one and two chondroitin sulphate chains, respectively, predominate (Waddington and Embery, 1991), primarily associated with the surface of mineral associated cells, pericellular areas, and on the borders and lumina of lucunae and canuliculi, although a smaller presence is indicated on or between the collagenous network within the mineralized matrix (Bartold, 1990; Smith *et al.*, 1996; Ababneh *et al.*, 1998, 1999). In contrast, the principle proteoglycans associated with the soft connective tissues of periodontal ligament and the gingival connective tissue are the dermatan sulphate containing forms of decorin and biglycan together with the larger chondroitin sulphate proteoglycan, versican (Rahemtulla, 1992).

The distribution of proteoglycans within the periodontium reflects the function of these macromolecules in the synthesis and remodelling of connective tissue. The SLRP decorin has been ascribed roles in regulating collagen fibril formation (Scott, 1988), regulating mineralization (Embery *et al.*, 1998) and, along with biglycan, in binding growth factors, TGF-β (Yamaguchi *et al.*, 1990). The predominance of chondroitin sulphate containing SLRPs in mineralized tissues and dermatan sulphate containing SLRPs in soft connective tissues may reflect their potential for inhibiting, controlling, or promoting the mineralization process (Embery *et al.*, 1998).

The presence of the larger chondroitin sulphate proteoglycan, versican (Figure 2) has been suggested to be involved in cell signalling, cell recognition and connecting extracellular matrix components with cell surface proteins. Recently versican has also been identified in osteoid, where it has been suggested to play a major role in the initial formation of the extracellular matrix, but is removed during matrix remodelling prior to mineralization (Robey *et al.*, 1993; Waddington and Langley, 1998).

Of the proteoglycans present on the cell surface, perhaps the most prominent members are those of the syndecan family. Structurally, these molecules consist of a protein core composed of an intracellular domain, a hydrophobic transmembrane domain, and an extracellular domain attached to which are heparan sulphate and or chondroitin sulphate glycosaminoglycan chains (Figure 2). Syndecans have been identified in multiple forms on the majority of cell and tissue types although their expression is selectively regulated in cell, tissue and developmental dependent manner (Kim *et al.*, 1994). This has led to a number of proposed functions including cell-cell, cell-matrix interactions and binding of a variety of growth factors, cytokines, and

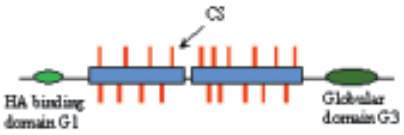
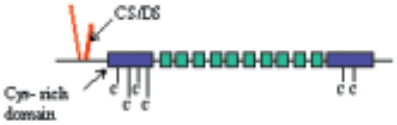
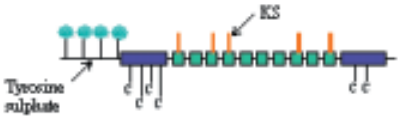
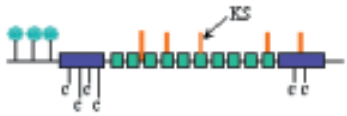
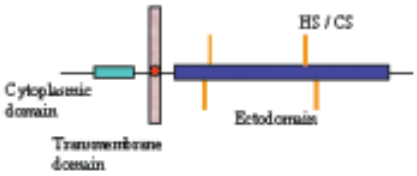
Name	Domain structure	Approx. monomer molecular size	Presence in periodontal tissues
Large aggregating Proteoglycans Versican		$1x10^6$ kDa	Soft connective tissues, periodontal ligament and gingiva; tentative identification in pre-mineralised tissues
Small Leucine-rich proteoglycans Decorin		2×10^5 kDa	DS substituted decorin and biglycan present in soft connective tissues, periodontal ligament and gingiva
Biglycan		3.5×10^5 kDa	CS substituted decorin and biglycan present in bone and cementum
Fibromodulin		1×10^5 kDa	Soft connective tissues periodontal ligament, gingiva, skin and cartilage. Identified in cementum.
Lumican		$7\text{-}8 \times 10^4$ kDa	Cornea and sorta. Recently identified in cementum
Cell Surface Proteoglycans Syndecan		$3\text{-}3.5 \times 10^4$ kDa	Syndecan-1 and -4 present on epithelial cells and fibroblasts; Syndecan-2 located on fibroblast cell surfaces

FIG. 2 The domain structure of the prominent proteoglycan present in periodontal tissues.

protease inhibitors, thereby influencing cell adhesion, differentiation and proliferation.

Along with proteoglycans, a number of other non-collagenous glycoprotein species and collagen forms represent important structural components. Glycoproteins collectively contain a protein core attached to which are N- and O- linked oligosaccharide chains, which may be branched or linear. As for proteoglycans, the distribution of glycoproteins reflects their function within the connective tissue. The most significant glycoproteins within periodontal ligament and gingival connective tissue are the adhesive glycoproteins, fibronectin, vitronectin, laminin, and thrombospondin. They exhibit several structural features, which include binding domains, allowing interaction of the various extracellular matrix proteins and cell binding sequences, such as Arg-Gly-Asp (RGD). As a consequence, adhesive glycoproteins have been assigned roles in cell attachment, cell behaviour, and formation of an organized extracellular matrix. In addition, glycoproteins specific to mineralized connective tissues have been reported. Of structural significance is the highly negative charge carried by these macromolecules, lending them to possible roles in controlling mineralization. Bone sialoprotein, osteonectin, and osteopontin contain highly conserved regions rich in acidic amino acids (reviewed by Bartold and Narayanan, 1998). The presence of sialic acid residues in bone sialoprotein and the potential for phosphorylation in osteopontin conveys further negativity, while the presence of RGD sequences allows for cell binding functions. Glycoproteins ostoecalcin and matrix Gla protein are two further anionic proteins by virtue of γ-carboxyglutamic acid residues.

The collagens represent a family of at least 19 differing species, which characteristically contain at least one stretch of the repeating amino acid sequence (Gly-X-Y), where X and Y are commonly proline and hydroxyproline. In acting as the principle structural component, the fibrillar collagens (type I, II, III, V, and XI) provide rigid structures conveying tensile strength and rigidity. Collagen type I present in mineralized connective tissues plays a major role in calcium phosphate crystal nucleation and deposition. Within connective tissues, collagen type I may represent some 60% of the organic components within the soft connective tissues of periodontal ligament and gingiva, and 90% of the total organic matrix of mineralized tissues, such as alveolar bone.

Extracellular matrix of the periodontium

For a comprehensive background to this topic the reader is referred to the review by Embery *et al*. (2000). What is clear from the outset is that the pattern of non-collagenous and collagenous molecules in the periodontium is similar to other soft and mineralized connective tissues present in the body. In relation to orthodontic movement the primary functional tissues are periodontal ligament and alveolar bone. The gingiva and underlying connective tissues do not have such an important value, and are therefore not included. In terms of extracellular composition, the components identifiable in gingival connective tissue are similar to those present in periodontal ligament, although differences in the molecular composition, arrangement, and metabolism of the individual collagenous and non-collagenous components is apparent, which is a reflection of the different functional loads placed on these tissues.

Periodontal ligament

The main collagen types are I and II with varying amounts of type V and XII also present. Type XII collagen expression is related to development, and patterns the organization and alignment of the periodontal ligament fibres. In addition, type VI collagen has been immunolocalized in both periodontal ligament and gingiva, and appears to be linked to both the small microfibrillar structures of collagen and elastin, and the highly ordered parallel arrays of oxytalin bundles. The arrangement of these collagen bundles is a vital feature in tooth movement and is the basis of a series of comprehensive reviews (Berkovitz *et al*., 1995).

The fibres are embedded within the non-collagenous matrix, the most studied components being the proteoglycans. The bulk of the studies have been directed at the glycosaminoglycan constituents and have included a wide range of types with dermatan sulphate predominating. Other predominant glycosaminoglycans include hyaluronan, chondroitin sulphate in an over-sulphated form and keratan sulphate. The periodontal ligament fibroblasts can synthesize these glycosaminoglycans in culture from where most data is derived (Larjava *et al*., 1992; Watanabe and Kubta, 1998).

The advent of specific antibodies to the proteoglycan species has permitted the identification of a range of proteoglycans in the periodontal ligament, including versican, a high molecular weight chondroitin sulphate proteoglycan, decorin, and biglycan, and a matrix-associated heparan sulphate proteoglycan (syndecan) and CD44 (Hakkinen *et al*., 1993).

A range of non-proteoglycan glycoproteins are also evident, and include fibronectin, laminin, vitronectin, thrombospondin and tenasin (Matsuura *et al*., 1995). Although the role of these molecules in the periodontal ligament is not established they would appear to confer cell interaction, regulation and attachment.

Alveolar bone

There is surprisingly little detailed information on the extracellular matrix of alveolar bone given its vital role in tooth movement and orthodontic therapy. It has an extremely high turnover rate (Sodek, 1977), and thus an active system of synthetic and degradative enzymes. The principle collagen identified is type I, with lesser amounts of type III identified (Becker *et al*., 1986). The predominant glycosaminoglycan identified is chondroitin sulphate (Waddington and Embery 1991). Dermatan sulphate and hyaluronan have also been identified in lesser quantities, and associated with the non-mineralized phase of the extracellular matrix. The proteoglycans are of low molecular weight, and support their identity as decorin and biglycan, a finding recently confirmed using polyclonal antibodies towards the small leucine-rich proteoglycans (Waddington and Embery, 1991; Moseley *et al*., 1998).

Chondroitin sulphate has been detected at the ultrastructural level using immunohistochemical procedures, and found to be located on both the cell surface and around the bone canaliculi and osteocytes (Bartold, 1990; Smith *et al*., 1996). A small chondroitin sulphate proteoglycan is present within the mineralized matrix.

Other non-collagenous molecules identified in alveolar bone are osteopontin and bone sialoprotein, which are expressed during the formation and remodelling (Matsuura *et al*., 1995). Of note is the localization of osteopontin at the ultrastructural interface between alveolar bone and periodontal ligament.

Cementum

The important role of cementum is the attachment of periodontal ligament. In common with periodontal ligament and alveolar bone, the principle collagen in cementum is type I with lesser amounts of types III, V, and VI (MacNeil and Thomas, 1993). Sharpey's fibres, which represent a major volume feature of cementum comprise mostly type I, with type III apparently coating type I collagen in these fibres.

The distribution of proteoglycan is primarily with the cementoblasts and cementocytes. The work of Cheng *et al*. (1996), using immunolocalization techniques indicated chondroitin sulphate/dermatan sulphate proteoglycan, and isolated using biochemical procedures the keratan sulphate rich proteoglycan, lumican, and fibromodulin. More recently, studies by Ababneh *et al*. (1998, 1999) have determined the presence of the hyaluronan-aggregating proteoglycan versican, decorin, and biglycan and keratan sulphate proteoglycan in cementocytes, and on the borders and lumina of lacunae and canaliculi in cellular cementum, in addition to cementoblasts on the root surface and in the periodontal ligament. Of note is the observation that proteoglycan and glycosaminoglycan were only apparently expressed by a limited proportion of cementocytes.

Cementum also contains cementum attachment protein, which is shown to promote adhesion and spreading of mesenchymal cell-types (Saito and Narayanan, 1999). Osteoblasts and periodontal ligament fibroblasts indicated more effective adhesion than gingival fibroblasts and keratinocytes. Fibronectin has also been shown to be an important component of mature cementum (Nishimura *et al*., 1989). Other adhesion proteins with RGD motifs are present in cementum, and include bone sialoprotein, osteopontin, osteonectin and osteocalcin, which regulates bone

mineralization and expressed by those cementoblasts which line the roots of developing and mature teeth (Nohutcu *et al*., 1997).

Remodelling of the extracellular matrix in bone and periodontal ligament

In considering the appearance of proteoglycans in GCF during orthodontic tooth movement it is also necessary to consider the underlying metabolic events, which occur within the tissues of the periodontium both during normal tissue remodelling and following application of an external force. All connective tissues within the body undergo a constant state of remodelling, synthesizing and degrading the macromolecular components of the extracellular matrix. The reason for this is in maintenance of structural integrity, which is of particular importance to the periodontal connective tissues in considering the stress, and strains placed on theses tissues. Indeed, periodontal ligament is one of the most highly metabolically active tissues in the body. *In vivo* radiolabelling studies of rats have identified half lives for the turnover of mature collagen in periodontal ligament was 2 days compared with 5 days for gingiva, 6 days for alveolar bone and 15 days for skin (Sodek, 1977).

More recently, specific consequences of extracellular matrix remodelling have been considered by Streuli (1999). Degradation of the extracellular matrix may therefore be considered important in influencing cellular function via altering cell-matrix interaction and thus cell signalling pathways. Moreover, in consideration of the interactive nature of macromolecules such as decorin and biglycan with growth factors such as TGFβ, specific degradation of these proteoglycans may lead to increased accessibility of the growth factor with the cell surface receptors. Partial degradation of the extracellular proteins may also result in conformational changes influencing cellular interaction or may lead to the release of bioactive peptides. Of note, a number of extracellular matrix components, such as versican and laminin, contain sequences homologous to epidermal growth factor, the proteolytic release of which may influence cellular activity (Yamada, 1992).

Remodelling of the extracellular matrix is also proposed to be important in control of the mineralization process during bone formation. It is generally well accepted that bone formation is accomplished by cells of the osteoblast linage, which progress through a series of maturational stages. In addition, the synthesis of the bone matrix proteins appears to be stage specific, with the secretion first of an unmineralized osteoid which is subsequently remodelled during mineralization. Although the function of the various proteins (as discussed above) is largely speculative, their temporal expression and structural features suggests that proteins expressed during early bone formation, such as versican, are implicated in dictating cellular function, the establishment of an extracellular matrix, and the inhibition of premature mineralization, which must be removed to allow for the deposition of calcium phosphate mineral crystal (Robey *et al*., 1993). Proteins synthesized at the mineralizing front, such as the bone sialoprotein, have proposed roles in osteoblast attachment and in co-ordinating mineral deposition, while the later expressed proteins such as osteocalcin possibly function in preventing hypermineralization and, via interaction with osteopontin, osteoclast recruitment (reviewed by Bartold and Narayanan, 1998).

A large number of enzymes are involved in the remodelling of the extracellular matrix. These include members of the serine proteases, aspartate proteases, and the cysteine proteases. However, the major candidates implicated in extracellular degradation are now considered to be the matrix metalloproteinase. A number of reviews provide details on the variety, activation, regulation and substrate specificities of this large family of endoproteases (for example, Corcoran *et al*., 1995; Kerrigan *et al*., 2000), which where initially broadly categorized into the collagenases, gelatinases, stromolysins, and membrane type matrix metalloproteinases. However, each matrix metalloproteinase type would appear to have its own spectrum of substrates, which may include both collagenous and non-collagenous components. Consequently, during tissue remodelling events, such as those seen during matrix formation and repair, the differential expression of matrix metalloproteinases, which is tightly controlled in a spatial and temporal fashion, is capable of producing the specific cleavage of the extracellular matrix proteins within localized tissue sites in co-ordinating these biological processes. The change in the activity and profile of the matrix metalloproteinases, together with the expression of their natural tissue inhibitor TIMP (tissue inhibitor of metalloproteinases) within the extracellular environment is therefore an important consideration in monitoring the metabolic activity of the periodontal tissues and in determining the pathways leading to the appearance of connective tissue metabolites in GCF. Changes in matrix metalloproteinase activity are likely during orthodontic tooth movement, which will be considered in a later section, and imbalances in the extracellular concentrations of matrix metalloproteinase and TIMPs have been cited as an important pathological mechanisms of connective tissue destruction in inflammatory diseases such as periodontitis (Reynolds and Meikle, 1997).

Response of the extracellular matrix in bone to stress

Remodelling of the extracellular matrix plays an integral role in orthodontic tooth movement with forces exerted on the tooth transmitted to the surrounding tissues of the periodontium. Classically, the movement of teeth has been explained via the pressure: tension hypothesis in which subjecting the periodontal tissues pressure results in bone resorption, while placing the periodontal tissues under tensile forces leads to bone deposition. However, studies examining the uptake and release of ^{3}H-thymidine and ^{3}H-proline by the periodontal ligament *in vivo* have indicated that metabolic activity was similar on both the pressure and tension sides of teeth subjected to an orthodontic force (Baumrind, 1969). The drawback of such experiments, however, is the inability to identify localized tissue remodelling. Immunolocalization studies have suggested a change in the profile of the proteoglycans within the periodontal ligament with an increased immuno-detection of chondroitin 6 sulphate epitopes near the bone surface corresponding to the compressive side during tooth movement (Kagayama *et al*., 1996). Earlier studies by Embery *et al*. (1987) have

also indicated changes to the extracellular matrix components in periodontal ligament placed under stress following intrusive loading of the teeth, with a reduction in the molecular weight of the proteoglycans compared with those extractable from undisturbed ligament. *In vitro* experiments on fibroblasts from various sources have indicated that flattened cells synthesize more DNA than rounded cell (Folkman and Mosconna, 1978), while cells induced to adopt a rounded shape by addition of phorbol esters are more catabolic than anabolic in their nature which increase expression of collagenase and plasminogen activator (Aggeler *et al*., 1984). This has led to the notion that cells in tension area are flattened, therefore inducing synthetic events, while cells in pressure areas are rounded resulting in degradation of the extracellular matrix.

However, when teeth are moved they may be displaced 10 times more than the reduction in the periodontal ligament suggesting bone is readily responsive to mechanical deformation (Baumrind, 1969). It is now well accepted that the osteoblast is pivotal to both the resorptive and formative phases of bone remodelling in response to mechanical stimuli, which is likely to be mediated by changes in the cytokine network influencing cellular activity (reviewed by Sandy *et al*., 1993).

Little or no specific information is available regarding the fate of the proteoglycan species present in bone during orthodontic tooth movement. However, changes to the collagenous components have been reported. Using a bone organ culture system, Meikle *et al*. (1984) have noted significant increases in collagen turnover in the tissue following applying tension to coronal sutures. In addition, the profile of the collagen appears to change with an increased synthesis of collagen type III noted. The reason for this change is not fully understood, but a number of studies have noted that collagen type III is present in significant quantities in foetal tissues, blood vessels, and periodontal ligament.

In considering the changes in the metabolic activity to the extracellular matrix so far reported following application of tensile mechanical stress to the tissues, changes in the activity of the matrix metalloproteinases have also been noted. Meikle *et al*. (1980) have reported an increase in the production of collagenases, gelatinases, and stromelysins, together with a corresponding increase in the overall concentration of TIMP within the coronal suture bone organ culture model. Subsequent, immunolocalization studies, however, have indicated that collagenase activity is localized, corresponding to sites of increased cell proliferation, and within these sites the concentration of TIMP is decreased (Meikle *et al*., 1980). Such changes in the matrix metalloproteinase activity indicate increased metabolism of the extracellular matrix in the presence of tensile stress in a tightly controlled fashion enabling increased remodelling of the periodontal tissues.

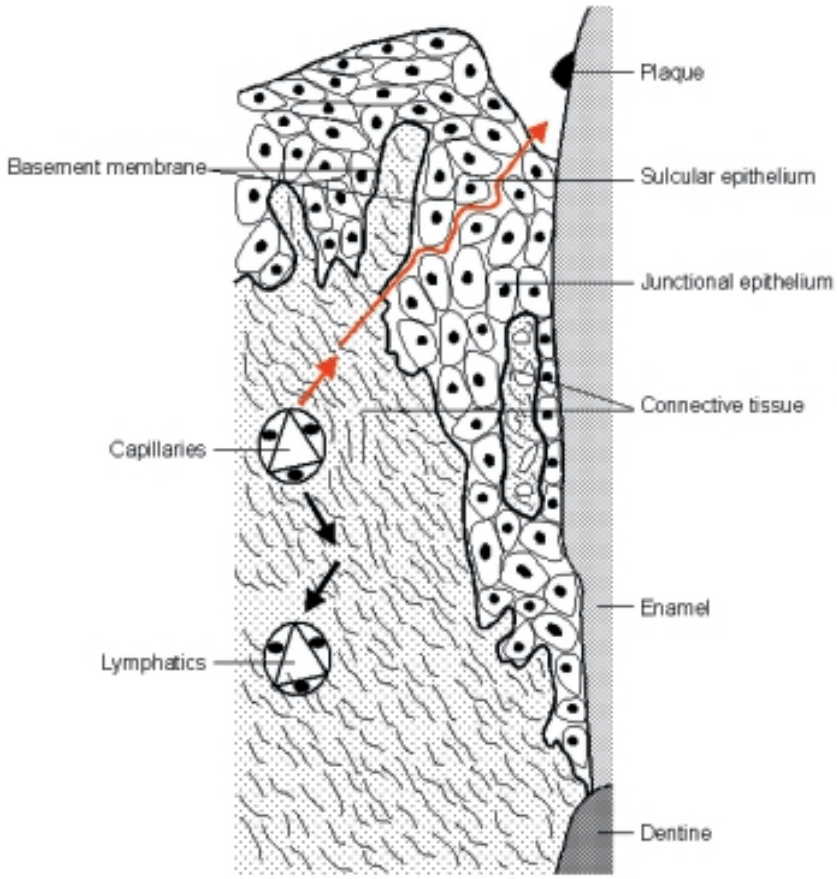


FIG. 3 The passage of gingival crevicular fluid originating from the vasculature of the deeper-seated connective tissues. The presence of plaque-derived particulate material within the gingival sulcus and the diffusion of macromolecules towards the basement membrane, leads to the establishment of an osmotic gradient along which fluid travels to appear as a transudate/exudate in the gingival crevice.

Gingival Crevicular Fluid

GCF represents a powerful fluid vehicle for the diagnostic clinician since it contains, depending upon the clinical situation an array of biochemical and cellular factors, which feature as biomarkers of the state of the periodontium. The early work of Embery *et al.* (1982), Last *et al.* (1985), and Waddington *et al.* (1994) have described the appearance and identification of a range of glycosaminoglycan, proteoglycan and tissue glycoproteins, which provide biochemical evidence on the underlying state of the biochemical tissues, which elude normal clinical parameters.

GCF can variously be described as a transudate or an exudate. It is a fluid that arises at the gingival margin and can be collected by a variety of procedures providing non-invasive, site-specific process. Its collection requires patience on the part of the clinician and ranges from the use of platinum loops, filter-paper strips, gingival washings, and the use of micropipettes, favoured by ourselves. The advantage of the latter procedure is ready storage, quantitation and application for either electrophoresis of direct chemical assessment.

The outcomes of a number of studies indicate that GCF is a feature of the fine nature of the gingival vasculature where the effect of trauma to the permeability of the arterial and venular capillaries leads to fluid production (Figure 3). In periodontal disease there is an increase in GCF volume, which clearly arises by breaching of the normal integrity conferred by the basement membrane and junctional epithelia. It has been established that this process is accompanied by enlargement of the intercellular spaces of the junctional epithelium and partial destruction of the basement membrane (Freedman *et al.*, 1968). Such events will lead to the production of a semi-permeable membrane and an osmotic gradient (Alfono, 1974). This will draw interstitial fluid from the surrounding capillaries and lymphatic system. The initial exudate is usually discarded, since it is not defined as an inflammatory exudate. However, with time a secondary inflammatory exudate, defined as GCF is evident and provides the basis for identification of constituent biomarkers, which represent the metabolic state of the underlying and deeper-seated tissues of the periodontium.

The value of GCF in the assessment of orthodontic movement will be governed by different parameters as essentially it will be non-inflammatory and non-plaque influenced. The resorptive/synthetic trauma on the deeper-seated tissues of the periodontium will induce a fluid pressure flow, which may be used to assess factors influencing orthodontic assessment. Evidence will be presented later to show that movement of alveolar bone and periodontal ligament induces the production of extracellular matrix factors for use as biomarkers of orthodontic treatment.

Detection of proteoglycan in GCF associated with orthodontic movement

The pioneer work on the elucidation of biomarkers of periodontal disease has been extended to the search for parallel markers of orthodontic movement. Emphasis has been directed at the proteoglycan and their constituent glycosaminoglycan.

The first report in this area was by Last *et al.* (1985), who detected chondroitin 4-sulphate in GCF on the side of a tooth towards which pressure was being directed by an orthodontic appliance. In a further study samples of GCF were collected simultaneously on the mesial and distal aspects of teeth undergoing a distal retraction movement by two experienced clinicians. When compared to control teeth a statistically significant raised level of chondroitin 4-sulphate was evident on the distal aspect (in which stress was being directed). From teeth at the retention stage raised amounts of this glycosaminoglycan were apparent at both surfaces.

Another glycosaminoglycan component detectable in GCF associated with orthodontic tooth movement is hyaluronan, which is an ubiquitous component of all GCF samples collected. No significant changes in the levels of this glycosaminoglycan were evident at the surfaces of the active and reduction groups.

As this orthodontic model is a non-plaque, non-disease related process it is tempting to suggest that the increased levels of chondroitin 4-sulphate represents biological alterations to the deeper-seated tissues of the periodontium notably alveolar bone. Although changes to the periodontal ligament cannot be ignored the absence of dermatan sulphate an important glycosaminoglycan component of PDL, makes such a possibility less obvious.

The same workers (Samuels *et al.*, 1993) carried out a longitudinal study of canine teeth undergoing retraction. An increase of chondroitin 4-sulphate occurred during the pre-treatment phase when the teeth were undergoing the most rapid movement in both horizontal and vertical modes. No significant increase in the same glycosaminoglycan were detected in similar teeth showing smaller horizontal-only or vertical-only movements with both fixed or functional appliances.

In a further study carried out longitudinally over 2 years (Pender *et al.*, 1994) the volume of GCF increased during active tooth retraction and showed a marked decrease in the retention stage in comparison to the pre-treatment stage. The orthodontic influence showed itself as an additional feature of GCF volume flows, even though gingival inflammation was evident throughout the period itself contributing to increased GCF flow in comparison to controls.

At the retention stage, elevated GCF volumes and glycosaminoglycans levels appeared to be related to a shorter duration of retention. A recent investigation of the early stages of orthodontic treatment revealed an increased GCF flow after 4 weeks of active movement and increased chondroitin 4-sulphate levels at the later stage of 10 weeks. Teeth that showed the greatest extent of movement, determined by reflex metrography, continued to show raised chondroitin 4-sulphate levels and GCF flow until 22 weeks, whereas chondroitin 4-sulphate levels declined in those teeth moving to a small extent. Such differences have been attributed to variations in the state of the deeper periodontal tissues at different stages of appliance therapy.

This group of related clinical studies on orthodontic treatment suggests that the chondroitin sulphate proteoglycan present in GCF represents a marker for active alveolar bone and periodontal ligament turnover. Biochemical characterization of proteoglycan metabolites in GCF during active orthodontic tooth movement has

revealed a composition of chondroitin sulphate components with a close similarity to those of human alveolar bone. As indicators of periodontal tissue changes, the volumes and chondroitin sulphate content of GCF samples represent a means of monitoring the responses to various types of orthodontic appliances at different stages of treatment that may be of value to the clinicians.

Detection of other extracellular components in GCF as diagnostic biomarkers

While the detection of proteoglycan metabolites in GCF has proved to be a potentially strong biomarker for assessing bone resorption and remodelling during orthodontic tooth movement, it may be possible to achieve a more detailed evaluation of the metabolic activity of the tissues through the examination of a range of biomarkers in GCF, including other connective tissue metabolites, enzyme activity, and cytokine levels. However, only a limited number of studies have addressed the value of other GCF constituents as indicators of tissue responses to orthodontic tooth movement.

The presence of the bone matrix protein, osteocalcin, has been identified in GCF associated with orthodontic tooth movement within adolescents (Griffiths *et al*., 1998). However, these studies suggested a high variability in levels of the protein between subjects, and a lack of a consistent pattern associated with the initial fitting of the appliance, retractive, and retentive stages of treatment, questioning its value as a biomarker for monitoring orthodontic treatment. The presence of osteocalcin in GCF may merely relate to the high levels of circulatory osteocalcin expected in adolescents where bone remodelling associated with the development of the skeleton and the dentition is very much apparent. Other potential matrix components used as markers of bone turnover in a variety of metabolic diseases, such as osteoporosis, include collagen cross-links pyridinoline and deoxypyridinoline, which are major components of mineralized connective tissues. However, and perhaps surprisingly considering the high level of bone metabolic activity, studies thus far have failed to detect the presence of these collagen cross links in GCF associated with orthodontic tooth movement (Griffiths *et al*., 1998).

More promising markers of monitoring orthodontic treatment are the changes in the profile, and levels of various cytokines and growth factors in GCF associated with the different stages of tooth movement. Several cytokines have now been identified as potent mediators of bone metabolism and include interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α), both of which have been implicated in stimulating bone resorption and inhibiting bone formation, along with interleukin-6 (IL-6), although its role is less clearly defined. A number of growth factors also display multiple effects on cellular activity in regulating bone remodelling and include most notably the members of the transforming growth factors (TGF), including TGF-α and the bone morphogenic proteins. During orthodontic tooth movement GCF levels of IL-1β, IL-6, and TNF-α< as well as the growth factors TGFβ and epidermal growth factor have all been observed to rise compared with contralateral control sites (Grieve *et al*., 1994; Uematsu *et al*., 1996). This pattern is also reflected in the analysis of levels of prostaglandin E, recognized as a pro-inflammatory mediator of bone metabolism, which is significantly increased following the initial onset of the application of a mechanical force (Grieve *et al*., 1994).

Studies by Insoft *et al*. (1996) have also attempted to relate activities of acid and alkaline phosphatase in GCF with bone metabolism associated with continued orthodontic treatment. Alkaline phosphatase was observed to peak during the first 3 weeks of treatment, while acid phosphatase was seen to increase over the subsequent 3–6 weeks following initiation of treatment. Of significance acid phosphatase is recognized as an important marker of osteoclast activity and bone resorption, whereas bone-specific alkaline phosphatase has been reported as a biomarker indicative of bone formation and both have been used in the monitoring serum and tissue samples in assessing of a range of bone diseases (Christenson, 1997). The presence of such markers in GCF may therefore be useful in identifying specifically osteoblastic and osteoclastic activity associated with bone remodelling during orthodontic tooth movement.

Concluding remarks

This review has provided details of the existing state of our knowledge on the utilization of biomarkers in GCF as a potential measure of orthodontic movement. It has highlighted the value of proteoglycans as salient biomarkers and research is now underway to modify their analysis on a small-scale required for chair-side diagnosis. Initial progress has been made to develop immunoassays procedures linked to the detection of the chondroitin sulphate proteoglycan decorin. It is also recognized that heparan sulphate appears in the GCF in certain clinical situations. This glycosaminoglycan is linked to the syndecan family of proteoglycans and may offer further avenues of research. It is not possible to ignore the potential role of other biomarkers of metabolic activity, which are beginning to provide an insight into the temporal aspects of the resorptive and formative processes occurring within the bone matrix during orthodontic treatment.

In conclusion, it may well be that a package of biomarkers may provide a more complete answer to diagnosis. Whatever the outcome, such data will provide the orthodontist with an extra means of assessing the metabolic events associated with orthodontic treatment, which cannot be obtained from current clinical indices.

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