

CHAPTER II

LITERATURE REVIEW

The review was divided into five parts as follows:

- I. Effects of orthodontic tooth movement on periodontium
- II. Gingival crevicular fluid (GCF)
- III. Glycosaminoglycans (GAGs)
- IV. Hyaluronic acid (HA)
- V. ELISA-liked assay for HA

I. Effects of orthodontic tooth movement on periodontium

Orthodontic tooth movement is involved with prolonged application of multidirectional forces on teeth. The tooth movement occurs as the alveolar bone (ALV) around the tooth remodels. The ALV is resorbed in some areas while deposited in the other. The tooth moves through the ALV carrying its attachment apparatus with it. The bony response is mediated by the periodontal ligament (PDL), so the tooth movement is primarily a PDL phenomenon. However, the changes in gingival tissue are also observed during orthodontic tooth movement (Ronnerman *et al.*, 1980).

Effects of orthodontic treatment on periodontal ligament

Forces exerted on a tooth produce a distortion of the PDL, resulting in alterations in cellular shape and configuration. This creates short-lived piezoelectric spikes that can lead to cellular activation by changing membrane polarity and ion channel activity, and also induces the releases of the neuropeptides from afferent nerve endings. Some of these neuropeptide molecules are vasoactive. This causes vasodilation and migration of leukocytes into the extravascular spaces. These migratory cells synthesize and secrete a wide variety of cytokines and growth factors.

In addition, as capillaries are stretched or compressed excessively, tissue damage may occur. Such events lead to the synthesis and the secretion of ECM components, tissue-degrading enzymes, acids, and local factors. This remodeling can cause cellular proliferation and differentiation and modification of the GCF compositions (Davidovitch, 1991; 1995).

Effects of orthodontic treatment on alveolar bone

Orthodontic tooth movement is associated with polarized changes in the periodontal tissues (Reitan, 1985). At the tooth surface towards which movement is directed, the resorption or compression side, the ALV resorption occurs in areas corresponding to compressed PDL fibers. At the opposite tooth surface, the deposition or tension side, the fibers in the PDL and the gingival tissues are subjected to tension and the deposition of the ALV occurs (Last *et al.*, 1988).

Many studies suggest that a raised level of GAGs, predominantly chondroitin-4-sulphate in the GCF collected at the compression side of a tooth during active tooth movement, may result from changes in the ALV and the PDL rather than in superficial gingival tissues (Samuels *et al.*, 1993; Last *et al.*, 1988; Kagayama *et al.*, 1996). Therefore, the GAG compositions in human ALV, like chondroitin-4-sulphate, present in the GCF are important as a prognostic indicator of deeper periodontal tissue turnover during orthodontic tooth movement (Waddington, 1989). However, the changes in HA levels may instead reflect superficial soft tissue remodeling, since HA is associated with the non-mineralized tissue of the ECM and can be identified in little amount of GCF during orthodontic tooth movement.

Effects of orthodontic treatment on gingiva

The gingival tissues are composed of epithelium and underlying connective tissue that are attached to the external part of the ALV and the supracrestal region of the tooth. The components of gingival tissues are fibers, ground substance or ECM, cells, and neurovascular elements. The ECM occupies the space between cells, fibers, and

neurovascular elements. Major constituents of ECM are water, glycoproteins, and proteoglycans (Yamalik *et al.*, 1998).

After orthodontic closure of an extraction site with fixed appliances, the gingival tissues on the mesial aspect of canine appears to be left behind as the tooth moves away from it. The epithelial attachment peels off from the tooth surface and a small red triangular patch appears adjacent to the mesial tooth surface (Artherton, 1970). On the distal aspect, the gingival tissue becomes hyperplastic where about 2 mm of the space is remained to be closed. Meanwhile, the teeth that are moving towards each other push the gingival tissue in front of them. As a result, a fold of epithelium and connective tissue is formed (Artherton, 1970; Ronnerman *et al.*, 1980). The histological and immunohistochemical analyses of this hyperplastic gingival tissue with invagination in the compression area demonstrate the presence of GAGs, especially the HA and the chondroitin sulphate (Ronnerman *et al.*, 1980).

II. Gingival crevicular fluid (GCF)

The GCF is a complex mixture of substances derived from serum, leukocytes, structural cells of the periodontium, and oral bacteria. The GCF is an osmotically-driven exudate found in the gingival sulcus. As an exudate, it tends to increase in volume with inflammation (Kavadia-Tsatala *et al.*, 2002). This process is caused by enlargement of the intercellular spaces of the junctional epithelium, and by partial destruction of basement membrane. Such events lead to the production of a semi-permeable membrane and an osmotic gradient.

The origin and the major constituents of the GCF have been the subject of many early studies. Recently, many investigations have been undertaken to study the association between the periodontal tissue changes and the flow rate, and the components of the GCF.

Effect of orthodontic treatment on GCF flow rate

The levels of the GCF components correlate with the clinical measurements of periodontal disease progression and with orthodontic tooth movement, so that the effect of orthodontic treatment itself on GCF flow rate cannot be determined unless periodontal diseases are eliminated (Kavadia-Tsatala, 2002). Many studies reported that the volume of the GCF has been increased during orthodontic treatment (Last *et al.*, 1988; Pender *et al.*, 1994; Baldwin *et al.*, 1999). In contrast, Uematsu *et al.* (1996) found that the volume of the GCF around the experimental teeth during orthodontic tooth movement was nearly the same as that around healthy teeth. Consistently, Miyajima *et al.* (1991) found no significant difference in GCF volume among treatment, retention, and control groups. Moreover, none of the patients had signs of gingival inflammation.

Effects of orthodontic treatment on GCF compositions

GAGs have been detected in GCF samples from sites around teeth, affected by chronic gingivitis, chronic periodontitis and juvenile periodontitis (Embery *et al.*, 1982; Last *et al.*, 1985), and from sites around endosseous dental implants (Last *et al.*, 1991). The pioneer work on the elucidation of biomarkers of periodontal disease has been extended to the search for markers of orthodontic tooth movement. Emphasis has been directed to the proteoglycans and their constituent GAGs (Waddington *et al.*, 2001).

Collection of GCF is a non-invasive and relatively simple procedure. Thus, GCF collection is an important method to analyze tissue status around teeth undergoing orthodontic movement. This may provide a useful tool for the modification of orthodontic treatment procedures (Kavadia-Tsatala, 2002).

III. Glycosaminoglycans (GAGs)

The GAGs contain one uronic acid (D-glucuronate or L-iduronate) and one sugar (N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc)). The GAGs, in the native state, are covalently linked to a core protein to form high molecular weight aggregates, called proteoglycans. The GAG chains are highly anionic linear chains.

The GAGs contain sulphate groups, with the exception of the HA that contains carboxyl groups. All GAG species form a highly negatively charged molecule under physiologic conditions (Bartold, 1987). The GAGs are localized on the cell surface or in the ECM. Along with the high viscosity of GAGs comes low compressibility, which makes these molecules ideal for a lubricating fluid and allows cell migration. At the same time, their rigidity provides structural integrity of both mineralized and non-mineralized connective tissue and cells (Last *et al.*, 1985; 1988; Samuels *et al.*, 1993; Kagayama *et al.*, 1996).

The GAGs in periodontium are HA, chondroitin-4-sulphate, chondroitin-6-sulphate, dermatan sulphate, heparan sulphate, heparin and keratan sulphate (Waddington, 2001). The chondroitin-4-sulphate rather than the chondroitin-6-sulphate isomer predominates in the gingival tissues, the PDL, the ALV, and the cementum. The dermatan sulphate and the heparin sulphate are also present in the gingival tissues and the PDL, but are not consistently found in the ALV. The HA is distributed throughout the periodontal tissues and is present in particularly high amounts in the gingival tissues (Embery *et al.*, 1979).

Fibroblasts are irregularly shaped cells responsible for the synthesis of various connective tissue fibers and the ground substance in which they are embedded. *In vitro* studies show that all GAGs identified in human gingival tissues are synthesized by fibroblasts (Bartold, 1987). The fibroblasts are also responsible for the removal of these structural elements. Therefore, the fibroblasts play a key role in maintaining and remodeling the connective tissue.

IV. Hyaluronic acid (HA)

The HA was first isolated from the vitreous humor of cattle eyes. The HA is one of the most important components of ECM of the subcutaneous tissues. The HA is a non-sulphated GAG that is non-covalently attached to a protein core to form proteoglycan (Waddington, 1989; 2001). It is a component of non-covalently formed complexes with proteoglycans in the ECM. The HA is a large polyanionic polymer being composed of

repeating 200-10,000 disaccharide units (N-acetyl-D-glucosamine and D-glucuronic acid) and its molecular mass can be up to 10 million Daltons (Ijuin *et al.*, 2001).

Due to its long, unchained polyanionic nature, the HA assumes a rather stiff, randomly coiled structure and can displace a large volume of water. The HA plays various biological and mechanical roles, such as resisting compression as a biological absorbent to mechanical stress, filling a large space for embryonic growth and development, repairing tissues, and creating a cell-free space into which cells eventually migrate (Bartold, 1987; Sato *et al.*, 2002).

Levels of the HA result from a balance between biosynthesis and enzymatic degradation. Recently, three human synthesized genes (HAS1, HAS2, HAS3) have been cloned and their features are elucidated. It is demonstrated that HAS1 and HAS2 synthesize high molecular-weight HA, whereas HAS3 generates low molecular-weight HA (Itano *et al.*, 1999). The HA levels decrease with cell differentiation. This permits compaction of tissue and allows cell to cell communication. The key event in the onset of differentiation appears to be the degradation of the HA by the concerted enzyme activities, such as endoglycosidase, hyaluronidase, and two exoglycosidases (β -glucuronidase and β -N-acetylglucosamidase). The hyaluronidase appears to be the most important enzyme. In wound healing process, the balance between HA deposition and hyaluronidase activity appears to be critical in modulating the various stages.

The HA levels are high during cell morphogenesis, but are decreased during cell differentiation. At this time there is a corresponding rise in hyaluronidase levels. The control mechanism for hyaluronidase has not been well elucidated. By extrapolation from other mammalian enzyme systems, it seems likely that the presence, or action, of hyaluronidase may be controlled by hyaluronidase inhibitors whose removal permits hyaluronidase activity to become manifest (Pogrel *et al.*, 2003).

Many studies suggest various roles of HA as follows:

1. Anti-inflammatory effect
2. Wound healing promotion
3. Biomarker for inflammation in the GCF
4. Reflecting the gingival tissue changes during orthodontic tooth movement

1. Anti-inflammatory effect

The HA has been suggested to have anti-inflammatory effects. If the HA is administered during surgery, scar formation is prevented. It influences and enhances tissue regeneration through its ability to retain large amounts of water (Ijuin, 2001). The HA is a GAG with anti-inflammatory and anti-edematous properties which are related to the osmotic activity (Jentsch *et al.*, 2003). The HA elevates, *in vitro*, the mesenchymal cell migration and differentiation (Pilloni *et al.*, 1992) as well as many functions of blood cells with special interest for the inflammatory response, e.g. phagocytosis and chemotaxis (Weigel *et al.*, 1986).

2. Wound healing promotion

Wound healing is a complex process, involving with interference by microorganisms, inflammatory components, and mechanical disturbances. In the oral cavity, the HA creates a local environment with a water binding effect, which may give an opportunity for osteoblasts to produce a matrix for wound healing (Engstrom, 2001). The concentration of the HA is increased during wound healing. High molecular weight HA stimulates early healing in rat long bones and reparative dentin formation (Sasaki *et al.*, 1995).

3. Biomarker for inflammation in the GCF

The HA has been studied as a metabolite or a marker of inflammation in the GCF. The concentration of the HA is increased in chronic gingivitis (Embery *et al.*, 1982; Last

et al., 1985; Smith *et al.*, 1997; Yamalik *et al.*, 1998). The correlation of the HA levels in the GCF with the gingival condition suggests that local degradation of the HA occurs in the GCF by enzymes from bacteria or lysosomes of host leukocytes. The absence of any detectable HA in exudates from sites of untreated acute ulcerative gingivitis appears to result from the increased levels of bacterial hyaluronidase (Last *et al.*, 1987).

Although it would seem likely that the HA content is influenced by changes in gingival inflammation, as observed *in vitro*, an increased synthesis of HA and a decreased production of chondroitin sulphate-containing proteoglycans by gingival fibroblasts from inflamed gingiva compared with those from healthy gingiva have been observed, but only a weak association has been found (Bartold, 1987).

4. Reflecting the gingival tissue changes during orthodontic tooth movement

Last *et al.* (1988) and Pender *et al.* (1994) found no significant increase in the HA levels from GCF around teeth undergoing active orthodontic treatment. During early retention phase, Last *et al.* (1988) also observed no significant increase in the HA levels. At the later stages of retention phase, a decrease in HA levels was even reported (Last *et al.*, 1988). Sato *et al.* (2002) found that the HA of both sides (compression and tension sides) did not change during experimental tooth movement and suggested that the HA had a very large molecular weight so it might not be affected by the orthodontic force.

However, Samuels *et al.* (1993) showed that the levels of the HA tended to be increased in all fixed appliance groups. No increase was found in the functional appliance group, possibly associated with the minimal effect of the functional appliances on the relative position of the canines in contrast to the fixed appliances. Moreover, the changes in HA levels strongly correlate with the increased GCF volume collected before tooth movement, but have no correlation during the treatment. Ronnerman *et al.* (1980) demonstrated an increased GAG content, predominantly HA, in human gingival tissues from the compression side of teeth during orthodontic movement.

V. ELISA-liked assay for HA

Almost all of previous studies were designed to quantify the changes of GAGs in the GCF by electrophoresis and laser densitometric scanning (Last *et al.*,1988; Pender *et al.*,1994; Samuels *et al.*,1993). This method is a lengthy procedure and requires the manipulations of samples. Therefore, this method may not be suitable to be a quick chair side method for the GAG quantification. In this study, we used an ELISA (Enzyme-linked Immunosorbent Assay) method, which could detect the GAGs in trace amounts from the GCF samples. Furthermore, the GAGs may be quantitatively analyzed more readily by the ELISA than by the electrophoresis method (Nishino *et al.*, 1990). Therefore, an ELISA is a suitable method for analyzing small amount of samples, a large number of samples, and several biomarkers in one sample simultaneously. This method can potentially developed to be a non-invasive quick chair-side diagnostic tool for Clinical Dentistry in the future.

The ELISA is a widely used method for measuring the concentration of a particular molecule, e.g., a hormone or drug, in a fluid such as serum or urine. The molecule is detected by antibodies that have been made against it, which is the antigen. This method is one of the immunoassays using two different antibodies reactive with the antigen whose concentration is to be measured. A fixed quantity of one antibody is attached to a series of replicate solid supports, such as plastic microtiter well plates. Tested solution containing antigen at an unknown concentration or a series of standard solutions with known concentrations of antigen are added to the wells and allowed to bind. Unbound antigen is removed by washing, and the second antibody, which is enzyme linked or radiolabeled, is allowed to bind. The antigen serves as a bridge, so the more antigens in the test or standard solutions, the more enzyme-linked or radiolabeled second antibody will bind. The results from the standards are used to construct a binding curve for the second antibody as a function of antigen concentration, from which quantities of antigen in the tested solutions may be inferred. When this test is performed with two monoclonal antibodies, it is essential that these antibodies see non-

overlapping determinants on the antigen; otherwise, the second antibody cannot bind (Abbas *et al.*, 2000). The advantages of the ELISA are similar to other antibody-labeled reactions, which include specificity, sensitivity, inexpensiveness, and safety. Since the enzyme labeling is the critical portion of the ELISA, its selection is very important. The enzyme selected should be stable under the conditions used for storage, cross-linking, and the assay.

With regard to the basic principle of our competitive-based ELISA assay, we have modified the conventional ELISA, which uses two specific antibodies that bind to the same antigen as described above, by substitution of antibodies with biotinylated hyaluronic acid-binding peptide (B-HABP) because HA is not a protein (or antigen), so the antibody cannot be raised against this heteropolysaccharide molecule. For a brief protocol of this assay, a fixed amount of the HA is immobilized on the solid support, and a fixed quantity of the HA specific indicator molecule is allowed to competitively bind to the HA. Similar to the labeled antibody, the B-HABP serves as an indicator molecule to bind competitively to the HA in the GCF samples, which subsequently decreases the amount of B-HABP available for binding to HA-coated wells. The specificity of the HA-HABP reaction appears to be high with a strong affinity approaching that of the avidin-biotin reaction (Stern *et al.*, 1996).