CHAPTER 2

REVIEW OF LITERATURE

The review is divided into four parts as follows:

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2.3 Gingival crevicular fluid (GCF)

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2.1 Orthodontic tooth movement and force magnitude

Orthodontic treatment is a method for correcting malocclusion by moving teeth toward proper positions through alveolar bone remodeling. In an extraction case, the extraction site in the premolar area is closed by canine movement using various mechanical and force delivery systems. Orthodontic tooth movement is a process of both pathologic and physiologic responses to externally applied force.[25] The applied force creates mechanical strains in the tooth-supporting tissues that can mainly be categorized as either compressive or tensile strains. The initiating inflammation at compressive sites is caused by constriction of the PDL microvasculature, resulting in a focal necrosis, hyalinization, and compensatory hyperemia in the adjacent PDL.[26] Focal hyalinization, which counteracts tooth movement, probably does not directly depend on the applied force but on local stresses and strains that may be quite individually and locally affected due to

irregularities in periodontal and bone morphology, thereby, facilitating individual differences in the rate of tooth movement.[27]

Orthodontic tooth movement has been defined as a result of the coupling of bone resorption at compressive sites and bone deposition at tensile sites adjacent to the PDL.[2] Extrinsic mechanical stimulation leads to remodeling of periodontal supporting tissues. Bone remodeling is a process, involving cellular functions directed toward co-ordinated resorption and formation of new bone, which can be regulated by systemic hormones and local factors.[28] Ideally, orthodontic tooth movement should provide a maximum rate of tooth movement with minimal and reversible damage to roots, PDL and alveolar bone. This can be achieved by creating an optimum orthodontic force magnitude, which efficiently moves teeth toward desired positions without causing discomfort or severe and irreversible tissue damage. It has been emphasized that light force application produces more tooth movement and less root resorption than does heavy force.[29] In 1932, Schwarz suggested that the optimal orthodontic force magnitude delivered to the tooth should be equal to the blood pressure of capillary vessels in order to prevent capillary vessel occlusion in the compressed PDL.[30] The optimal force magnitude for bodily tooth movement (translation) ranges from 70 to 120 grams and it should differ for each tooth and for each particular patient.[31]

A number of experiments that studied the relationship between force magnitude and rate of tooth movement have been conducted in animals,[29,32,33] with a minor proportion of reported studies in human subjects. Those experiments varied in species, range of force magnitude, the specific teeth chosen for the experiment, direction of tooth movement, duration of experiment, and force reactivation interval,

but no evidence-based optimal force level could heretofore be recommended in clinical orthodontics.[34]

Boester and co-workers compared the rates of canine movement among four different force levels (55, 140, 225, and 310 grams), and reported that the 55 grams of force level produced a significantly slower rate of canine movement than 140, 225, or 310 grams, whereas the latter three force levels produced similar rates of canine movement.[35] After that, Owman-Moll and co-workers reported no significant difference in the rate of tooth movement when forces of 50 centiNewton and 100 centiNewton were applied for buccal tipping of premolars,[36] and that the rate of tooth movement was increased up to 50 per cent when a force of 200 centiNewton was applied in comparison to a force of 50 centiNewton.[37]

Moreover, various experiments have been designed for moving canines into premolar extraction spaces. Some experiments used nickel-titanium closed coil springs with a force of 100 grams,[38,39] and some used elastomeric chains exerting an initial force of 250 grams.[5,7,10]

Yee and co-workers measured rates and amounts of orthodontic tooth movement under heavy (300 grams) and light (50 grams) continuous forces. It was found that the heavy force increased the rate and the amount of canine movement. However, adverse effects, such as loss of canine rotation control and of anchorage, were prominent. Light force produced a greater rate of canine retraction than did heavy force, with less strain on anchorage.[40]

2.2 Assessments of orthodontic canine movement

1. Clinical assessment

Tooth position after orthodontic treatment can be clinically assessed either by comparison between pre- and post-treatment dental casts or by intra-oral measurements using a ruler, a caliper or a divider. A slide caliper, a co-ordinate measuring machine and laser measuring equipment based on holograms have been used to measure horizontal tooth movement.[41] Additionally, the distance of canine movement can be determined in the transverse, sagittal and vertical planes of space by a reflex metrograph (Ross Instruments Ltd., Salisbury, U.K.) on model casts from upper and lower alginate impressions taken before the commencement of active treatment and at the end of treatment.[19]

Moreover, three dimensional finite element methods have been used to simulate orthodontic canine movement using sliding mechanics[42] and using a canine retraction spring.[43] Orthodontic canine movement has also been biomechanically assessed by comparing the amounts and directions of movement as measured by the finite helical axis system and the rectangular coordinate system.[44]

2. Radiographic assessment

The amount of canine movement can be assessed by measurement on radiographs before and after the treatment using superimposition of pre- and post-retraction lateral cephalograms.[35,38]

3. Biochemical assessment

Biochemical assessment is a method for assessing periodontal activity during orthodontic tooth movement using biochemical constituents of GCF. Orthodontic tooth movement leads to periodontal and alveolar bone remodeling, and this process

can be assessed by monitoring changes in the levels of GCF constituents. GCF is an inflammatory exudate, which can be easily collected from the gingival crevice of a tooth using a paper strip or micropipette. This method is non-invasive and can detect periodontal responses in the early phase of tooth movement.

2.3 Gingival crevicular fluid (GCF)

GCF is an exudate that arises toward the gingival margin between the tooth surface and the epithelial integument or within the gingival crevice. GCF consists of substances derived from serum, bacteria, leukocytes, activated epithelial cells, connective tissue cells and bone cells. The initial product of healthy GCF is the transudate of gingival tissue interstitial fluid, produced by an osmotic gradient that induces the flow of interstitial fluid from the connective tissue to the gingival sulcus. In gingivitis or periodontitis, the transudate is transformed into inflammatory exudates or true exudates of GCF.[45] The physical, protective effects of GCF are flushing of the pocket and facilitation of the passage of antibacterial substances. The production of GCF results from increasing permeability of blood vessels beneath sulcular and junctional epithelia.

The GCF flow rate and composition vary depending on the condition of the periodontal tissues. In patients with gingivitis or periodontitis, the GCF volume is increased.[11,46-49] Furthermore, during orthodontic treatment, the force produces distortion of the ECM in the PDL and movement of tissue fluid, resulting in alterations in cellular shape and cytoskeletal configuration. These processes are reflected in the synthesis and secretion of ECM components, tissue-degrading enzymes, acids, and inflammatory mediators in deep periodontal tissues, resulting in

modification of both GCF flow rate and its compositions.[50] Many studies have shown that an increase in the GCF volume is associated with orthodontic tooth movement.[15,17,19]

There are various methods for collecting GCF.[50] Microcapillary tubes are used collect GCF by placing them at the gingival crevices, and either holding them at a particular site or continuously removing them from the gingival crevices and returning them for 10 to 15 minutes. This procedure can often disrupt delicate crevicular epithelium, resulting in contamination of native GCF with blood and serum, and can also cause a dilution of the native GCF by an influx of serum from the capillary vessels in the gingiva. The GCF can also be collected by placing a prewashed absorbent string into the gingival crevices. This method can also disrupt the epithelium and involve problems with the accurate weighing of small samples. Another common method for collecting GCF is a placement of filter paper strips into the gingival crevices. This method causes less disruption of the crevicular epithelium and enables more rapid measurements of the GCF, thereby decreasing the probability of contamination of GCF with serum. Additionally, a paramagnetic bead method has been used as an alternative procedure for isolating and purifying tumor necrosis factor (TNF) from the GCF. This method does not require collecting any fluid from the gingival crevices. The beads, covered with anti-TNF monoclonal antibodies, are placed into the gingival crevices, where the antibodies form complexes with the TNF; then, they are retrieved with a special magnetic harvester.

2.4 Biomarkers in GCF

GCF is a source of biomarkers used for assessing metabolic changes in the periodontium. Therefore, GCF constituents have been used as prognostic and diagnostic markers for periodontal disease and for biologic activity in the periodontium during orthodontic tooth movement.

Biomarkers in GCF are divided into four categories as follows:[51]

1) Products derived from subgingival microbial plaque

Chronic periodontitis is primarily the result of a finite number of bacterial species within a complex biofilm on the subgingival surface of teeth. The main bacteria implicated are anaerobic and gram-negative bacteria, which include *Porphyromonas gingivalis, Bacteroides forsythus, Prevotella intermedia, Aggregatibacter actinomycetemcomitans, Capnocytophaga species, Treponema denticola* and other spirochaetes, *Fusobacterium nucleatum, Campylobacter rectus and Eikenella corrodens*.[52] All of the periodontopathic bacteria in the gingival crevice or periodontal pocket produce both proteolytic and hydrolytic enzymes into the GCF, both of which play a role in periodontal pathology by either reducing the effectiveness of the host defenses or degrading the host tissue.[53]

2) Inflammatory mediators

Orthodontic tooth movement induces, in periodontal tissue, an acute inflammatory response, which consists of local ischemia, vasodilatation and migration of inflammatory cells through the periodontal capillaries. Inflammatory cells produce and release cytokines as inflammatory mediators for regulating bone remodeling. The inflammatory mediators include interleukin-1β (IL-1β), tumor necrosis factor-α

(TNF-α), prostaglandin E_2 (PGE_2), substance P (SP), transforming growth factor (TGF), serum antibody, total protein concentrations and acute-phase proteins.

Interleukin-1 (IL-1) is a cytokine which initiates bone resorption. It exists in two forms: alpha (IL-1α) and beta (IL-1β). Both IL-1α and IL-1β are produced by macrophages, monocytes, fibroblasts, and dendritic cells. The biologic activity in the periodontium during orthodontic tooth movement is reflected by IL-1β, SP and PGE_2 levels in GCF.[6] The levels of SP and IL-1β in GCF are increased during orthodontic canine movement in adults, and are involved in periodontal inflammation in response to mechanical stress.[7] IL-1β and IL-8 levels in GCF are significantly elevated during orthodontic canine movement.[54] Grieve and co-workers[55] reported that $PGE_{1/2}$ and IL-1β levels in GCF were significantly higher than in control teeth during orthodontic treatment. Elevation of TNF-α levels in GCF has also been reported during orthodontic tooth movement.[4] In addition, the concentrations of IL-1β, IL-6, TNF-α, epidermal growth factor, and beta 2-microglobulin have been shown to be elevated in GCF during orthodontic canine movement.[5]

The TGF-β family plays important roles in growth and development, in inflammation, and repair (including angiogenesis) and in regulation of host resistance mechanisms.[56] TGF-β1 regulates a broad range of biological processes, including cell proliferation, cell survival, cell differentiation, cell migration, and production of ECM. It is one of the most important factors in the bone, helping to maintain the balance between the dynamic processes of bone resorption and bone deposition.[57] It is also associated with the bone remodeling during orthodontic canine movement.[58]

3) Host-derived enzymes

Host-derived enzymes include alkaline phosphatase, β-Glucuronidase, cysteine proteinases, cathepsin, collagenases, elastase, neutral proteolytic enzyme, gelatinase, and tartrate-resistant acid phosphatase or acid phosphatase 5, tartrate resistant (TRAcP or ACP5).

Alkaline phosphatase is an enzyme found in many cells of the periodontium, including osteoblasts, fibroblast, and neutrophils, and is involved in bone metabolism. Some forms of alkaline phosphatase are produced by plaque bacteria. Phosphatase activity in GCF is useful for monitoring tissue responses to orthodontic treatment.[8] The activity of alkaline phosphatase in GCF reflects biologic activity in the periodontium during orthodontic movement; thus, it should be further investigated as a diagnostic tool for monitoring orthodontic tooth movement in clinical practice.[9] Therefore, alkaline phosphatase activity can be a biological indicator of periodontal activity and orthodontic tooth movement.[39]

β-Glucuronidase is a lysosomal enzyme found in the primary granules of neutrophils. The elevated levels of β-Glucuronidase in GCF, indicative of increased activity of polymorphonuclear leukocytes, are associated with probing attachment loss in patients with chronic periodontitis.[59]

Cysteine proteinases, a group of degradative enzymes, present in multiple forms in numerous tissues of animals and plants as well as in microorganisms. One type has been implicated in bone degradation, including cathepsin B, cathepsin L, cathepsin S and the novel cathepsin K. Cathepsins are proteolytic enzymes, found in animal tissue, that catalyze proteins into polypeptides. Higher levels of cathepsin B, H and L activities have been found at sites with more serious signs of disease activity

in patients with periodontitis.[60] Orthodontic tooth movement also induces an increase of cathepsin L in GCF as a result of extracellular matrix degradation in response to mechanical stress.[10] Cathepsin K is a lysosomal cysteine protease involved in bone remodeling and resorption, and being predominantly expressed in osteoclasts. During tooth movement, cathepsin K is synthesized by odontoclasts on the pressure side and secreted into the tooth resorption lacunae. Therefore, cathepsin K may take part in the degradation of the dentin matrix (type I collagen fibrils and non-collagenous proteins) of the tooth root.[61] It also acts as a biochemical parameter to monitor peri-implant tissue health.[62]

Collagenases are members of the matrix metalloproteinase family, which are produced by a variety of cells, including neutrophils, macrophages, fibroblasts, keratinocytes, and osteoclasts. Crevicular fluid collagenase activity can be used as an indicator of periodontal health and periodontal disease status.[11]

Neutrophil elastase is a serine proteinase stored in the azurophil granules of polymorphonuclear leukocytes. Crevicular fluid elastase levels are significantly correlated with gingival index, bleeding index, probing depth, and the amount of attachment loss.[63] Neutrophil elastase also acts as biomarker of bone loss around dental implants.[64]

Acid phosphatases are a family of enzymes, which belong to the hydrolase class. They have different forms which can be found in different organs, and their serum levels are used as a diagnostic tool for disease in the corresponding organs. Isoenzymes of acid phosphatases differ widely regarding tissue and chromosomal origin, molecular weight, amino acid homology, sequence length, and resistance to L(+) tartrate and to fluoride.[65]

Tartrate-resistant acid phosphatase or acid phosphatase 5, tartrate resistant (TRAcP or ACP5) is a glycosylated monomeric metalloenzyme expressed in mammals and highly expressed by osteoclasts, activated macrophages, dendritic cells, and a number of other cell types. It is synthesized as a latent pro-enzyme and activated by proteolytic cleavage and reduction. It is localized within the ruffled border area, lysosomes, Golgi cisternae, and vesicles.[66] Two forms of TRAcP circulate in human blood; TRAcP 5a derives from macrophages and dendritic cells, and TRAcP 5b derives from osteoclasts.[67] In a previous *in vitro* study, mature osteoclasts responded to mechanical strain by significantly increasing mRNA expression of TRAcP and cathepsin. Those results suggest up-regulation of bone resorption by osteoclasts directly from mechanical stimulation.[68] Furthermore, TRAcP has been proposed as a histochemical marker for osteoclast identification.[69-71] However, expression of TRAcP is an important biomarker in bone pathology not only for histochemical identification of osteoclasts but also as a serum marker of osteoclast activity and bone turnover.[72-74] Elevation of the levels of serum TRAcP have been detected in diseases characterized by increased bone resorption, such as postmenopausal osteoporosis,[75] hairy cell leukemia,[76] multiple myeloma,[77] and metastatic malignancies involving bone resorption.[78]

4) Tissue breakdown products

Tissue breakdown products include proteoglycan, GAGs, hyaluronic acid, fibronectin, osteocalcin, osteopontin, osteonectin, procollagen, laminin, and hemoglobin ß-chain peptide.

Proteoglycans in GCF are biomarkers during orthodontic tooth movement, notably proteoglycans associated with active resorption of alveolar bone.[18]

Proteoglycan metabolites have been used as biomarkers for assessing the metabolic changes in periodontal tissue and as a diagnostic tool in monitoring orthodontic tooth movement.[79]

GAGs are large complexes of negatively charged heteropolysaccharide chains, generally associated with a small amount of protein. These compounds have the special ability to bind large amounts of water, thereby producing the gel-like matrix that forms the basis of the body's ground substance. GAGs stabilize and support cellular and fibrous components of tissue, and help maintain the water and salt balance of the body. As essential components of the extracellular matrix, GAGs play an important role in mediating cell-cell interactions. GAGs are classified, according to their monomeric compositions, type of glycosidic linkages, and degree and location of their sulfate units, as hyaluronic acid, heparin and heparan sulfate, keratan sulfate, dermatan sulfate, chondroitin 4- and 6-sulfate.[13] GAG levels of peri-implant crevicular fluid are an effective marker for measuring and maintaining changes in bone metabolism.[80] The GAG components in GCF, particularly CS, reflect changes in deep periodontal tissues of alveolar bone and periodontal ligament during orthodontic treatment.[17] The changes of CS levels in GCF act as a biomarker of deep periodontal tissue changes during orthodontic tooth movement.[16] Chondroitin-4-sulfate is used as a potential diagnostic aid of periodontal tissue destruction.[81] Additionally, the expression of C-6-S near the bone surface is associated with the compressive force during tooth movement.[20] A previous study showed that the detectable C-6-S levels in GCF were associated with the applied orthodontic forces during orthodontic canine movement. Therefore, C-6-S is used as a biomarker for alveolar bone remodeling in orthodontics.[24] Furthermore, CS (WF6 epitope) levels

can be detected in peri-miniscrew implant crevicular fluid (PMICF) around miniscrew implants; therefore, CS (WF6 epitope) levels are used as biomarkers for assessing alveolar bone remodeling around miniscrew implants during orthodontic loading.[82]

Hyaluronic acid is a non-sulfated glycosaminoglycan found in gingival tissue. It can be detected in GCF in association with chronic gingivitis. A previous study showed that the cyclical pattern in the change of hyaluronic acid did not correlate with the force applied to canines, so hyaluronic acid can not be used as a reliable marker for changes in alveolar bone and periodontal tissue during orthodontic treatment.[83]

Fibronectin is a large group of heterogenous glycoproteins, and is present in blood and connective tissues. The role of fibronectin is to serve important cell-binding functions during wound healing, phagocytosis, and cell migration. Crevicular fluid fibronectin is partially degraded both in periodontal health and disease, and the degree of degradation of fibronectin decreases with periodontal treatment and increases with periodontal inflammation.[84]

Osteocalcin or Bone Gla protein is one of the major noncollagenous proteins of bone, which is synthesized by osteoblasts. It has been reported that the presence of osteocalcin in GCF reflects the degree of periodontal inflammation[85] and can be considered as a potential marker of periodontitis.[12]

Osteopontin is a non-collageneous protein of bone matrix produced by ostoblasts and also found in kidney, blood, mammary gland, and salivary glands. It relates to bone resorption as well as to bone formation, and also increases at the stage of matrix maturation and mineralization in ostoblast differentiation and in bone resorptive sites. The progression of periodontal disease is related to increasing osteopontin levels in GCF.[86]

Laminin is a 900-kDa glycoprotein, which is found in all basement membranes. It influences cell differentiation, migration, and adhesion. Higher amounts of laminin in GCF from patients with periodontitis, suggest the presence of hyperactive neutrophils during the transmigration process through the endothelium/epithelium.[87]