CHAPTER 2

LITERATURE REVIEW

The review is divided into five parts as follows:

2.1 Biomechanics for canine movement

2.2 Orthodontic elastomeric materials

2.3 Nickel-Titanium closed coil springs

2.4 Assessments of canine retraction

2.5 Gingival crevicular fluid (GCF) and enzyme-linked immunosorbent assay (ELISA)

2.1 Biomechanics for canine movement

The extraction of first premolars as a practical form of orthodontic therapy has been accepted for many years. Many types of malocclusion were corrected by this technique; extraction of either mandibular first premolars or second premolars, and maxillary first premolars for treatment Class II division 1 malocclusion (Al-Nimri, 2006), four first premolar extractions and retraction of the anterior teeth for treatment bimaxillary dentoalveolar protrusion (Bills *et al.*, 2005; Chae, 2007) and four first premolar extractions for crowding treatment (Gianelly, 1994). The procedure after first premolar extraction in orthodontic treatment are usually followed by canine retraction.

Orthodontic canine movement requires a force-delivery system which should meet the following criteria: 1) provide optimal tooth movement to elicit the desired effect, 2) be comfortable and hygienic to the patient, 3) require minimal operator manipulation and chair time, 4) require minimal patient cooperation, and 5) be economical. Numerous force-delivery systems have been proposed to fulfill these criteria. These include intra-arch and inter-arch force systems, coil springs, latex elastics, elastic threads, sectional arch wire auxiliaries and elastomeric auxiliaries (Sonis *et al.*, 1986).

Orthodontic canine movement by conventional fixed appliance therapy normally utilizes sliding mechanisms for space closure. The widely used force-generating materials are either elastomeric chains or Nickel-Titanium closed coil springs (Samuels *et al.*, 1993b; Samuels *et al.*, 1998; Dixon *et al.*, 2002; Hayashi *et al.*, 2004; Jaito *et al.*, 2006; Robert *et al.*, 2006; Leethanakul *et al.*, 2008; Poolkerd *et al.*, 2009; Intachai *et al.*, 2009).

Many studies have compared the efficiency of interrupted orthodontic force pattern generated by elastomeric chains to that of continuous force pattern generated by Nickel-Titanium closed coil springs during orthodontic tooth movement both *in vitro* and *in vivo* (Samuels *et al.*, 1993b; Samuels *et al.*, 1998; Dixon *et al.*, 2002; Santos *et al.*, 2007; Leethanakul *et al.*, 2008).

A recent *in vitro* study, designed to compare the forces generated by four commercially available elastomeric chains and Nickel-Titanium closed coil springs, was established to determine force decay pattern of these materials (Santos *et al.*, 2007). Nickel-Titanium closed coil springs showed initial force values closer to the ideal, and presented minimal force decay over 28 days. The elastomeric chains generated higher initial force values than those generated by the Nickel-Titanium closed coil springs, and presented higher force decay within the first 24 hours. The results of their study may imply that Nickel-Titanium closed coil springs should be more appropriate devices for space closure in orthodontic than elastomeric chains.

Four *in vivo* studies have compared the efficiency of elastomeric chains to that of Nickel-Titanium closed coil springs for orthodontic canine movement. The main factor that these studies used as an indicator of materials' efficiency was the rate of canine movement (Samuels *et al.*, 1993b; Samuels *et al.*, 1998; Dixon *et al.*, 2002; Leethanakul *et al.*, 2008).

Samuels *et al.* (1993b, 1998) serially compared the rates of bilaterally extracted first premolar space closure by Nickel-Titanium closed coil springs (150 gm) and by elastic modules (generating initial force of 400-450 gm), and concluded that; 1) Nickel-Titanium closed coil springs had a significantly greater and more consistent rate of space closure than did elastic modules, 2) there was no clinically observable difference in the tooth positions between the two techniques, 3) there was no evidence of greater patient discomfort with the springs.

Samuels *et al.* (1998) examined the rate of bilaterally extracted first premolar space closure using either 100 or 200 gm of force generated by Nickel-Titanium closed coil springs.

The conclusions from the first and the second studies are as follows: 1) Nickel-Titanium closed coil springs, for all magnitudes of force, produced a more consistent rate of space closure than did the elastic modules, 2) the 150 and 200 gm springs produced a faster rate of space closure than did the elastic module or the 100-gm springs, 3) no significant difference was noted between the rates of closure for the 150 and 200 gm springs.

In 2002, Dixon *et al*. (2002) designed a randomized clinical trial to compare three methods of orthodontic space closure: active ligatures, polyurethane elastomeric chains and Nickel-Titanium closed coil springs. Elastomeric chains were stretched double length of their resting lengths, while Nickel-Titanium closed coil springs were generated initial force as 200 gm. From their results, they concluded that Nickel-Titanium springs gave the most rapid rate of space closure and were considered to be the treatment of choice, but these appliances are high-cost materials. Elastomeric chains provided an economical treatment option that was as effective, but was likely to take more chair time for replacement at each visit.

Leethanakul *et al.* (2008) compared the efficiency of a continuous orthodontic force pattern to that of an interrupted orthodontic force pattern for orthodontic maxillary canine movement by using biochemical assessment. They investigated the effects of continuous and interrupted force on interleukin-1ß and interleukin-8 levels in human GCF. Their study used 20 orthodontic patients (14 females and 6 males: 13-23 years). Nickel-Titanium closed coil springs and elastomeric chains were activated, by stretching, and calibrated before placement, to producer approximately 6 ounces (about 170 cN) of initial force magnitude. Elastomeric chains were changed but Nickel-Titanium closed coil springs were not changed, unless it showed signs of distortion or force decay. GCF was collected from the distolabial side of the maxillary canine of each quadrant five times: before bracket placement, before canine retraction, 24 hours, one month, and two months after force application. IL-1ß and IL-8 levels with both force patterns showed significant elevation at 24 hours, and then declined. IL-1ß and IL-8 levels with Nickel-Titanium closed coil springs were higher than those with elastomeric chains at 24 hours, and one and two months after force application. In addition, the rate of canine movement with Nickel-Titanium closed coil springs was faster than that with elastomeric chains.

However, Lee *et al.* (2004) evaluated the effects of a light continuous force generated by Nickel-Titanium closed coil springs and an interrupted force generated by a screw-attached retractor by monitoring prostaglandin E_2 (PG E_2) and IL-1ß levels in GCF during maxillary canine retraction. They reported that when a continuous force was applied, the levels of both biomarkers were elevated at 24 hours and then decreased. When an interrupted force was applied and reactivated weekly, the levels of IL-1ß were elevated at 24 hours and a significantly greater elevation occurred during 24 hours after the first appliance reactivation, while the levels of PG E_2 increased significantly at 24 hours and remained high for 1 week. In addition, efficiencies of tooth movement produced by both force patterns were not significantly different. Furthermore, Nightingale and Jones (2003) compared the rate of maxillary anterior teeth contraction between Nickel-Titanium closed coil springs and elastomeric chains. They reported that both appliances closed space at a similar rate.

In several studies related to orthodontic tooth movement, the initial force magnitudes of Nickel-Titanium closed coil springs and elastomeric chains varied.

Ren *et al*. (2003) systematically reviewed both human and animal studies which were found in Medline and by hand-searching of major orthodontic and dental journals for the optimum force magnitude for orthodontic tooth movement, and identified 305 articles on human studies from 1952 to 2000 AD. After applying exclusion criteria, 12 of 305 articles on human studies remained. Eight studies involved canine retraction. A wide range of initial forces (18–1500 cN) was used in these studies.

After 2000 AD, Nickel-Titanium closed coil springs were used in several studies to generate force during orthodontic canine movement. Jason *et al*. (2009) compared the rate of tooth movement under heavy (300 gm) and light (150 gm) continuous orthodontic forces. It was concluded that initial tooth movement would benefit from light force, and that heavy force tended to increase the rate and the amount of canine retraction but caused unwanted clinical side effects, such as anchorage loss and loss of canine rotation control.

Jaito *et al.* (2006) used Nickel-Titanium closed coil springs with 125-140 gm of force during orthodontic maxillary canine movement and used biochemical assessment of chondroitin-6-sulfate levels in gingival crevicular fluid around these canines to evaluate the periodontal response.

Badri *et al.* (2008) compared the amount of anchorage loss of the molars, and the rate of canine movement with and without the use of implant anchorage during canine retraction. Closed coil springs (100 gm of force) were used.

Intachai *et al.* (2009) used 50 gm of force generated by Nickel-Titanium closed coil springs for maxillary canine retraction.

Poolkerd *et al.* (2009) studied the effects of orthodontic elastomeric chains during canine retraction *in vivo*. In their study, a split mouth design, they used two commercial elastomeric chains (Tuff® and Dynaflex®) for retracting maxillary and mandibular canines. Two brands of elastomeric chains were stretched 70-75% of the actual canine retraction distance. The mean value of initial force generated by Tuff® was 320 gm, and that by Dynaflex® was 290 gm.

In many studies of orthodontic canine movement by Nickel-Titanium closed coil springs (Samuels *et al.*, 1993b; Samuels *et al.*, 1998; Dixon *et al.*, 2002; Jaito *et al.*, 2006; Badri *et al.*, 2008; Leethanakul *et al.*, 2008; Intachai *et al.*, 2009; Jason *et al.*, 2009), the initial force magnitude for Nickel-Titanium closed coil springs ranged from 50 – 200 gm. In three studies of orthodontic canine movement by elastomeric chains (Samuels *et al.*, 1993b; Leethanakul *et al.*, 2008; Poolkerd *et al.*, 2009), however, the initial force magnitude for elastomeric chains ranged from 170 – 400 gm. Ideally, to compare the biochemical effects of orthodontic force patterns on periodontal tissue response during orthodontic canine movement, similar initial force magnitudes should be selected for Nickel-Titanium closed coil springs and elastomeric chains (Leethanakul *et al.*, 2008). Not only should the selected force magnitude not be so low as to render the elastomeric chains non-functional, but also it should not be so high as to cause unwanted side effects from continuous heavy force. The optimal range of force for bodily movement (translation) has been established as 70 to 120 gm (Proffit, 2007). So, the selected force magnitude for canine movement in this study was 120 gm.

2.2 Orthodontic elastomeric materials

Elastomeric materials are amorphous polymers made from polyurethane. Elastomeric chains were introduced for orthodontic tooth movement in 1970 (Andreasen *et al.*, 1970).

Elastomeric chains fulfill orthodontic requirements as follows: 1) provide optimal tooth moving force, 2) are comfortable and hygienic to the patient, 3) require minimal operator manipulation and chair time, 4) require minimal patient cooperation, and 5) are economical (Sonis *et al.*, 1986).

Many studies of elastomeric chain properties and their application for orthodontic practice have been published since 1970. Those studies examined force delivery, degradation properties and the effects of prestretching.

Force delivery and degradation properties

Andreasen and Bishara (1970) compared latex elastic and elastomeric chains while simulating intra-arch space closure by measuring intra-arch force. It was concluded that, after 24 hours, elastomeric chains lost 74% of their force, whereas latex elastic lost only 42%. However, the remaining force for the next three weeks of elastomeric chains was greater than that for latex elastic when stretched to the same distances. Later, Hershey and Reynolds (1975) compared three different samples of elastomeric chains. There were substantial differences in the initial force delivery of the chains, and they suggested that a force gauge should be used before applying elastomeric chains.

Wong (1976) studied the chains from two manufactures and showed that initial force loss of 50% occurred in the first three hours for both chains. Moreover, Lu *et al.* (1993) compared force decay curves of three types of elastomeric chains and reported that force loss of 41% occurred at the first hour and 67% at the end of the 4th week during their study period.

Ash and Nikolai (1978) compared the force decay of chains extended and stored in air, in water, and *in vivo*. They reported that chains exposed to an *in vivo* environment exhibited significantly more force decay after 30 minutes than those kept in air. No difference was noted between the chains stored in water and those stored *in vivo* until 1 week. Force decay was proved to be more rapid in water and *in vivo* than in air. In addition, Huget *et al.*, 1990 reported that water absorption of elastomeric chains and concurrent formation of hydrogen bonds between the water molecules and macromolecules of the elastomers are the causes of force degradation.

De Genova *et al.* (1985) investigated force degradation of chains from three companies, maintained at constant length and stored in artificial saliva. In the first of two studies, one set of specimens was maintain at 37°C and another was thermocycled between 15° and 45°C. They reported that the thermocycled chains displayed significantly less force loss after three weeks. The second study compared the force decay rate of thermocycled chains held at constant length to those subjected to simulated tooth movement of 0.5 mm per week. The chains subjected to tooth movement retained 9% to 13% less force than those at constant length. Besides, results of their study also showed that short filament chains generally provided higher initial force levels and retained a higher percentage of the remaining force than did long filaments. The force decay of the chains was in the range of 50% to 75%.

Jose *et al.* (2006) determined force-decay levels of elastomeric chains made by different fabrication procedures, injection molded and die-cut stamped elastomers. The results revealed no statistically significant difference in the force decay between elastomeric chain types during the three-week study period. The mean remaining force (about 150 gm) after three weeks was considered clinically adequate for canine retraction. They concluded that both types of elastomeric chain produced similar clinical effects.

Poolkerd *et al.* (2009) studied two commercial elastomeric chains (Tuff® and Dynaflex®) for canine retraction. Both chains were stretched 70-75% of the actual of canine retraction distance. Thirty two subjects were treated with four first premolar extractions. Two brands of chains were used randomly in different arches and quadrants for each patient. The subjects were divided into four groups and generated force was measured at initial placement and after 1 hour, 7, 14 and 28 days for each group, respectively. The results showed that the mean force delivery of both chains were in the proper ranges for canine retraction (350-100 gm through 28 days).

Effect of pre-stretching

Wong (1976) recommended pre-stretching elastic chains a third of their original length to pre-stress the molecular polymeric bonds in order to improve the strength of the chains. Young and Sandrik (1979) rapidly pre-stretched two types of elastomeric chains to pre-determined distance and placed the chains on a holding device designed to load them 90 gm. The controls of this study were non pre-stretched elastomeric chains. After 24 hours' immersion in 37 ° C water, one of the products exhibited 17% to 25% increased retention of force delivery capability, whereas the other chain showed no change. They repeated this study by increasing initial force to 180 gm, resulting in a greater decay of force than the control. However, the results revealed that one type of tested elastic showed a significant increase in force loss while the others were unaffected.

Kyung-Ho *et al.* (2005) evaluated the effects of prestretching on time-dependant force decay of five-unit (12.5 mm) and six-unit (15.5 mm) synthetic elastomeric chains from four manufactures. The chains were prestretched 100% for one hour (n =12), 24 hours (n =12), two weeks (n =12), and four weeks (n =12) in 37° C distilled water. The prestretched and unprestretched (control) modules were then stretched to 30 mm in 37° C water, and their forces were measured at initial placement, one hour, 24 hours, and weekly for four weeks with a digital force gauge. Results showed that the effects of prestretching on the force decay of elastomeric chains were noted mainly in the first hour. Thus, the clinical value of prestretching a synthetic elastomeric chain was questionable.

2.3 Nickel-Titanium closed coil springs

Nickel-Titanium closed coil spring was introduced for orthodontic tooth movement in 1986. Its special characteristics were super-elasticity and shape memory effect. Super-elasticity, sometimes called pseudoelasticity, is an elastic (reversible) response to an applied stress, caused by a phase transformation between the austenitic and martensitic phases of a crystal. It is exhibited in shape memory alloys. Pseudoelasticity is derived from the reversible motion of domain boundaries during the phase transformation, rather than just bond stretching or the introduction of defects in the crystal lattice. Shape memory effect refers to the ability of the material to "remember" its original shape after being plastically deformed while in the martensitic form (Fujio *et al.*, 1986).

Nickel-Titanium alloy exhibits a specific stress-strain curve and delivers a constant force over an extended portion of the deactivation range and exerts a very long range of constant light and continuous force (Fujio *et al.*, 1986; 1988).

Force delivery of Nickel-Titanium closed coil springs varies in response to factors as follows (Tripolt *et al.*, 1999):

1) Amount of its activation: The standardized springs, a 15-mm activation, followed by a 7.5-mm deactivation to the desired activation of 7.5 mm, deliver a relatively constant force if the spring is used for 5 mm of tooth movement.

2) Oral temperature: Super-elastic coil springs are extremely temperature sensitive and thus produce a large force variation at different mouth temperatures. However, in a narrow temperatures range, this variation is small.

2.4 Assessments of canine retraction

Assessment of canine retraction can be performed by various methods.

1) Clinical assessment

Clinical assessment for orthodontic canine retraction includes rate of canine movement, canine position after retraction and patient discomfort evaluation.

Rate of canine movement

The rate of canine movement is derived from actual canine retraction distance per unit of time. Several authors have selected various reference points for calculating the rate of canine movement in their studies. Samuels *et al.* (1993b; 1998) used the distance from a point between the central incisors to a clear, reproducible landmark on the first molar in each quadrant. Dixon *et al.* (2002) used the distance between the cusp tip of the tested canines to the buccal groove of the first permanent molar in all four quadrants, whereas Leethanakul *et al.* (2008) used the distance between the distal contact of the canine and a line perpendicular to the median reference line (the mid palatal raphe) through the medial end of the third palatal rugae in each quadrant. In addition, Robert *et al.* (2006) measured the distance from the cusp tip of the maxillary permanent canine to the facial cusp tip of the maxillary second premolar with a digital caliper. The mean rate of canine movement in their study was 1.3 mm per month.

Jason *et al.* (2009) compared the rate of tooth movement under heavy and light continuous orthodontic forces. Intraoral and maxillary cast measurements were made at the beginning of canine retraction (T0) and every 28 days for 84 days (T1, T2, T3) to assess total space closure. They used stable palatal reference points and the tips of the canines and the cusp tips of the first molars. Then, they scanned the model and printed the resulting image, and made linear measurements. The initial casts for each patient were used as the baseline model, with an acetate template for linear and angular measurement constructed according to the stable reference points (medial and lateral ends of the third palatal rugae) and superimposed on the subsequent cast images for measurement of canine and molar movement, and canine rotation. The results showed that the initial tooth movement (T0-T1) was not related to force magnitude but during T1-T2 and T2-T3, heavy force had a higher rate of tooth movement than did light force. However, heavy force tended to produce unwanted clinical side effects such as canine rotation and loss of anchorage.

Canine position after retraction

Shpack *et al.* (2008) studied the duration and anchorage management of canine retraction with translation and tipping mechanics. Assessment of the final position of the retracted canine was measured from dental casts using the angle formed between a line through the distal and mesial contact points of the canine, and the midpalatal raphe. The results showed that tooth angulations produced by both types of mechanics were significantly different. The mean tooth angulation produced by tipping mechanics was higher than that produced by translation mechanics.

Jason *et al.* (2009) compared the rates of tooth movement under heavy and light continuous orthodontic forces. In their studies, the side effects of canine retraction (distobuccal rotation) were measured from the angle formed between a line connecting the contact points of the mesial and distal surfaces of the canines from the superimposed templates representing the original and the final tooth position.

Patient discomfort evaluation

Samuels *et al.* (1993b) evaluated patients' discomfort from the use of Nickel-Titanium closed coil spring and elastomeric chains for canine retraction, and reported that there was no difference in patient discomfort between the two devices for canine movement.

2) Radiographic assessment

Robert *et al.* (2006) used mini-implant anchorage for maxillary canine retraction. The initial root parallelism of the maxillary canines in relation to the permanent lateral incisors and second premolars was compared with the root parallelism after retraction. Canine retraction on each side was categorized by the investigator as translation, slight tipping, or excessive tipping. The results showed that 57% of canines were bodily moved, 29% were slightly tipped and the last 14% were excessively tipped.

Hayashi *et al.* (2004) compared maxillary canine retraction using sliding mechanics and that using a canine retraction spring and analyzed the results by using a three-dimensional analysis based on a midpalatal orthodontic implant. A 3-D surface-scanning system was used to measure the series of dental casts. The results showed that both distal movement of the crown and canine tipping produced by the two methods were not significantly different.

3) Biochemical assessment

The analysis of specific constituents of GCF may provide quantitative biochemical indicators for local cellular metabolic activity (Delima *et al.*, 2003). The components of GCF, which are used as biomarkers for periodontal disease and periodontal tissue response under orthodontic forces, are divided into four categories as follows (Delima *et al.*, 2003; Giannobile *et al.*, 1993):

1. Products derived from subgingival microbial plaque
2. Inflammatory mediators including interleukin-1ß, prostaglandin E2, serum antibody, total protein concentration and acute-phase protein.

3. Host-derived enzymes, including alkaline phosphatase, ß-galactosidase, collagenase, neutral proteolytic enzyme, elastase and gelatinase.
4. Tissue-breakdown products, including glycosaminoglycans (GAGs; such as chondroitin sulfate, dermatan sulfate), fibronectin, osteopontin, osteonectin, procollagen, laminin and hemoglobin ß-chain peptide.

In the orthodontic field, the major part of research on tooth movement in humans must be performed in patients with normal, healthy gingival tissue and good control of oral hygiene. For this reason the literature review showed that products derived from subgingival microbial plaque were rarely used as biomarkers for periodontal tissue response under orthodontic forces.

The periodontal ligament cells respond to mechanical stimuli, including orthodontic forces, by releasing cytokines and growth factors that trigger the biological processes associated with alveolar bone resorption and apposition (Meikle, 2006). The level of inflammatory mediators found in GCF is believed to play an important role in the pathogenesis of the periodontium (Delima *et al.*, 2003). For this reason, various inflammatory mediators have been used as biomarkers for orthodontic tooth movement. However, in orthodontic research related to levels of biomarkers in GCF during orthodontic tooth movement, if the subjects are unable to control their oral hygiene suitably, inflammation of periodontal tissue produced by gingival plaque may be critical disturbing factors for those studies.

Host-derived enzymes, including acid and alkaline phosphatases, glycoprotein-degrading enzymes, proteinases, and enzymes associated with tissue destruction, in GCF have been studied as biomarkers for periodontal metabolism (Embery *et al.*, 1994). However, host-derived enzymes can be used as direct biomarkers for bone resorption closely less than the fourth category of biomarkers, tissue-breakdown products.

The response of periodontal ligament and alveolar bone to orthodontic force causes degradation of extracellular matrix. The degradation products leak into the GCF (Delima *et al.*, 2003). One element of the extracellular matrix is proteoglycans. Proteoglycans are comprised of a protein core to which one or more glycosaminoglycan chains are covalently attached. Chondroitin sulfate (CS) is the predominant type of glycosaminoglycan detected and represented in alveolar bone (Waddington *et al.*, 1989). It can represent the degenerative change of the deeper periodontal tissue of alveolar bone (Kavadia-Tsatala *et al.*, 2002; Waddington *et al.*, 2001). The presence of chondroitin-6-sulfate (C-6-S), one subtype of chondroitin sulfate, has been associated with applied compressive force during orthodontic tooth movement (Kagayama *et al.*, 1996). The study of Last *et al.* (1995) concerning glycosaminoglycan in GCF during orthodontic tooth movement showed a significant rise in chondroitin sulfate levels in the GCF of the teeth undergoing orthodontic tooth movement. Jaito *et al.* (2006) used a newly synthesized WF6 monoclonal antibody (which represents the degenerative epitope of chondroitin-6-sulfate) and an enzyme-linked immunosorbent assay (ELISA) method to detect chondroitin sulfate levels as a biomarker for alveolar bone remodeling, and reported that the detectable chondroitin sulfate levels were associated with the applied orthodontic forces.

2.5 Gingival crevicular fluid (GCF) and enzyme-linked immunosorbent assay

GCF is a fluid that emerges between the tooth surface and the gingival lining epithelium. It plays a protective role around the sulcular region, due to the flushing effect and transportation of antibacterial substances, either of host origin or those introduced into the circulation, such as antibiotics, to the crevicular space. Some types of irritation, chemical or mechanical stimulation, and chronically inflamed gingivae can induce the production of GCF as an exudate by increasing the permeability of the blood vessels underlying the junctional and sulcular epithelium (Egelberg, 1966).

GCF flow rate and components have been used as indicators for gingivitis and periodontitis. Pander *et al.* (1994) found that the GCF volume collected from the site of the greatest gingival imflammation was higher than that collected from the sites of lesser inflammation. Brecx *et al.* (1987) reported the correlation between GCF flow rate and histological changes during gingivitis. Last *et al.* (1988) and Baldwin *et al.* (1999) reported a significant increase of GCF volume during orthodontic tooth movement.

Application of orthodontic force to a tooth induces the movement of periodontal tissue fluid, the strain in cells and in extracellular matrix, and is followed by local damage of periodontal ligament and by tissue remodeling (Kavadia-Tsatala *et al.*, 2002). GCF components are also changed. Many GCF components, such as interleukine-1ß, prostaglandin E2, substance P, tumor necrosis factor -α (TNF-α), glycosaminoglycans and chondroitin sulfate, are used as biomarkers to evaluate cellular response under orthodontic loading (Dudic *et al.*, 2006; Jaito *et al.*, 2006; Kavadia-Tsatala *et al.*, 2002; Last *et al.*, 1995).

GCF assessment is a non-invasive investigation (Kavadia-Tsatala *et al.*, 2002). The methods for collecting GCF can be performed as follows:

1. Placing the microcapillary tube into the gingival crevice for 10 to 15 minutes. This technique may disrupt the crevicular epithelium and causes the contamination of GCF by blood and serum.
2. Using a prewashed absorbing string
3. Placing filter paper strips in the gingival crevice. This technique is a common method for collecting GCF; it causes less disruption to crevicular epithelium than other techniques, and decreases the contamination of GCF by serum.
4. Using a para-magnetic bead method. The GCF is not removed from the crevice, but the bead covered by monoclonal antibody is placed in the sulcus and GCF is analyzed by a special magnetic harvester. This technique is especially used for detecting tumor necrosis factor (TNF).

The methods for analysis of GCF include electrophoresis (Samuels *et al.*, 1993a) and enzyme-linked immunosorbent assay (ELISA) (Shibutani *et al.*, 1993).

ELISA can be divided according to its process techniques into four types :

1) Indirect ELISA, 2) Sandwich ELISA, 3) Competitive ELISA, 4) Reverse ELISA

For competitive ELISA, the major advantage of this type of ELISA is the ability to use crude or impure samples and still selectively bind any antigen that may be present. The steps for this ELISA are

1. Unlabeled antibody is incubated in the presence of its antigen.
2. These bound antibody/antigen complexes are then added to an antigen coated well.
3. The plate is washed, so that unbound antibody is removed. (The more antigen in the sample, the less antibody will be able to bind to the antigen in the well, hence "competition.")
4. The secondary antibody, specific to the primary antibody is added. This second antibody is coupled to the enzyme.
5. A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal.

Generally, for detecting chondroitin sulfate, monoclonal antibody (mAb) 3B3 and 9A2 have been used (Shibutani *et al.*, 1993). But recently, a newly synthesized monoclonal antibody, WF6, against the degenerative epitope of chondroitin sulfate was used by Jaito *et al.* (2006) in collaboration with the Thailand Excellence Center for Tissue Engineering, Department of Biochemistry, Faculty of Medicine, Chiang Mai University. This monoclonal antibody is against chondroitin sulfate and the detection of chondroitin sulfate represents a metabolic change in alveolar bone during orthodontic tooth movement. So the chondroitin sulfate (WF6 epitope) levels in GCF around the mandibular canines retracted by either a continuous or an interrupted force pattern generated by Nickel-Titanium closed coil springs or elastomeric chains, respectively, should be monitored during orthodontic canine movement.