CHAPTER V

DISCUSSION

The results from this study showed the presence of HA, which is a non-sulphated glycosaminoglycan, in GCF samples from both experimental canines and control incisors. The HA was found in all treatment phases, i.e. before, during, and after complete orthodontic canine movements, consistent with the findings from previous studies (Last *et al*., 1985; 1988). Previous studies have shown the association between different types of GAGs present in GCF and changes in the periodontal tissue (Fine, 1986; Curtis *et al*., 1989). In particular, there were changes in the HA levels found in GCF in association with orthodontic tooth movement by the fixed appliances, but not the functional appliances (Samuels *et al*., 1993). This may be because the fixed appliances cause much more localized changes around the teeth than the functional appliances, resulting in a greater distance of tooth movement (Samuels *et al*., 1993). Similarly, the changes in the levels of chondroitin sulphate, another type of GAGs, were shown to be correlated with vertical tooth movement in the same study (Samuels *et al*., 1993). In addition to the high HA levels detected in GCF during the phase of tooth movement, there was another study (Ronnerman *et al*., 1980) that showed a correlation of high levels of HA present in the gingival tissue at the distal surface of canines during the retention phase. This also confirms the essential role of HA during orthodontic tooth movement. Therefore, it is possible that orthodontic tooth movement, particularly with fixed appliances, may result in changes in the HA levels in GCF, which are indicative of connective tissue turnover (Samuels *et al*., 1993). This was an underlying reason for conducting this study.

The results from our study showed a cyclical pattern of HA changes which occurred in every treatment phase (the leveling, the movement, and after complete orthodontic canine movement phase). This pattern has never before found in other

previous studies (Ronnerman *et al*., 1980; Last *et al*., 1988; Samuel *et al*., 1993; and Pender *et al*., 1994). This may be due to the different designs between our study and theirs, i.e. the frequency of GCF sample collection or different study designs. In our longitudinal study, we collected GCF samples much more frequently than the other studies (Last *et al*., 1988; Samuel *et al*., 1993; and Pender *et al*., 1994). In addition, we studied the HA changes in the same individual throughout our longitudinal study. This would avoid the subject variations in terms of HA quantities seen in a cross-sectional study done by Last *et al*. (1988). Interestingly, the cyclical pattern of HA changes found in this study was not predictable and appeared not to be associated with the timing when a force was applied to the canines. The possible association between HA level changes and the treatment time was tested by the non-parametric statistical analysis, i.e. Friedman Test, and the result showed no statistically significant differences in terms of HA levels per unit protein in any periods of treatment. Consistently, a large variation in the duration from 2 to 10 weeks between the two highest neighboring HA levels observed in this study also confirms the unpredictable changes in HA levels.

It was interesting to note that the HA levels at pre-orthodontic appliance placement, or T0 phase, were considerably high in some teeth, while those in the other teeth remained low. This may be due to differences in the oral health and hygiene of each individual subject at that time. Therefore, we suggest that the baseline HA levels of normal population be estimated before conducting this type of study in the future.

The cyclical pattern found in the leveling phase might be related to the force used to align the teeth before canine tooth movement (Samuels *et al*., 1993). Consistently, the HA level changes could be detected in this phase. In addition, the high levels of HA detected in the initial leveling phase of orthodontic appliance insertion might be due to increased inflammation around the periodontal tissue (Embery *et al*., 1982; Last *et al*., 1985; Smith et al., 1997; Yamalik et al., 1998) from the reduced ability of the new patients to clean their own teeth after having bulky appliances in the mouth.

The variations in HA levels were also observed in the movement (M) phase in each patient as well as in each tooth. A lack of consistent pattern of changes in HA levels in this phase suggests that HA level changes are not correlated with the applied force from a closed coil spring to retract the canine. It is possible that other factors besides the force from the closed coil spring influence changes in HA levels in GCF collected from retracted canine in this M phase.

During a two-month period after completing canine movement, or in the S phase, the high HA levels and the cyclical pattern of HA level changes, both increased and decreased HA levels, were also observed similar to the cyclical pattern in the M phase, although the closed coil spring was removed. This may be because the force from a ligature wire that tied the canine with the second premolar in order to maintain the canine position to prevent relapse. Otherwise, the force originating from a new position of the canine, e.g. from occlusal contacts, surrounding muscles, and stretched collagenous fibers, especially the fibers of the supracrestal gingival group, results in continuous changes in superficial periodontal tissue that cause the cyclical pattern of HA level changes observed in the S phase. Furthermore, unlike alveolar bone and periodontal ligament that regain their original structure in the remodeling process, several studies have shown that the gingival tissue does not regain its pretreatment structure, but it is compressed and consequently retracted in response to the orthodontic tooth movement (Artherton, 1970; Ronnerman *et al*., 1980; Redlich *et al*., 1999). This would cause the continuous stress and strain in the superficial gingival tissue, leading to the HA level changes even in the after complete orthodontic canine movement phase. These factors in conjunction with the persistence of the marked localized changes in the deeper periodontal tissue induced by orthodontic tooth movement are likely to play a role in the gingival tissue remodeling, leading to continuous changes in HA levels even in this retention phase (Linhe *et al*.,1982).

Each tooth had several highest HA levels throughout the study. The duration between the two highest neighboring HA values varied greatly, ranging from 2 to 10

weeks. This varying pattern confirms our suggestion that the HA level changes are not correlated with the applied force from a closed coil spring, and that the HA levels in human GCF is not a good "biomarker" for monitoring changes during orthodontic tooth movement.

Comparisons of the HA levels per unit protein between canines and incisors in any point of GCF collection in every treatment phase by the Mann-Whitney U test showed that there were no significant differences in HA levels between canines and incisors. This is consistent with the lack of association found between the HA level changes and the applied force in the canine. Surprisingly, the HA levels in the incisors were relatively high in some points, and the cyclical pattern of HA level changes in incisors was similar to that in canines although there was no direct force exerting on the incisor. It was possible that the force from closed coil spring affected the main arch wire that might be transferred to the incisors. The size of main arch wire used in the M phase was 0.016x0.016'' in the brackets that had the size of slot around 0.018x0.025''. Therefore the main arch wire was smaller than the size of slot and perhaps it was too small to minimize canine tipping. This resulted in canine tipping and caused the main arch wire to bow. As a result, the pressure caused by the bowed arch wire would affect the incisors to undergo indirect vertical movement. The vertical movement of incisors is clinically seen by the characteristic of anterior deep bite in the incisors that is often found in an orthodontic patient treated with the coil spring (Gjessing, 1992; Dincer *et al.,* 2000). Therefore, this indirect pressure on the incisors might explain the detectable levels of HA in GCF and the changes in HA levels in the incisors. On the other hand, there might not be the indirect force from the closed coil spring affecting the incisors as mentioned above, but the detectable HA levels or the cyclical changes in HA levels might result from the possible traumatic occlusion from masticatory force due to the characteristic anterior deep bite in the incisors (Linhe *et al*., 1982).

The GAG compositions of the periodontal tissue usually indicate the possible origins of the GAG type detected in GCF. It has been shown that HA comprises

approximately 17% of the total GAGs found in the gingival tissue (Embery *et al.,* 1979; Bartold 1986; 1987), and only a minor component in bone, periodontal ligament, and cementum (Pearson *et al*., 1975; 1982; Bartold *et al*., 1988; Waddington *et al*., 1989). Consequently, the relative bulk and high contents of HA in gingival tissue suggest that the gingival tissue is a main source of HA detected in GCF. However, it is possible that a minor component of HA in alveolar bone, periodontal ligament, and cementum can add up to the total HA levels in GCF as well. Interestingly, the cyclical pattern of HA level changes in this study was similar to that of other bone markers in association with bone resorption and formation during orthodontic tooth movement, such as acid and alkaline phosphatase, respectively (Insoft *et al*., 1996), as well as pro-inflammatory cytokines, one of the first biomolecules involved with inflammation that always happens in an early stage of tissue remodeling (Uematsu *et al*., 1996). Therefore, it is suggested by these studies that multiple cycles of bone remodeling, a coupled process in which there is localized removal of old bone (resorption) and replacement with newly formed bone (formation), eventually be harmonious with soft tissue remodeling during the tooth movement, resulting in the cyclical HA changes in harmony with the bone cycles. This association is remained to be investigated in the future longitudinal study with an increased sample size.

A previous study has shown that degeneration of the gingival tissue by chronic gingivitis can result in an HA increase in GCF, since HA is a major component in the gingival tissue (Last et al., 1985). In addition to the increase in HA levels by tissue degeneration, increased synthesis of HA by gingival fibroblasts isolated from inflamed gingival tissue as compared with those isolated from healthy gingival tissue has been observed *in vitro* (Bartold, 1987). Therefore, it is not known whether the periodic increases in HA levels observed in our study are from the gingival tissue degeneration or the increased HA synthesis by inflamed gingival fibroblasts. However, Embery *et al*. (1978) found that the total amounts or proportions of HA extracted from both non-inflamed and severely inflamed human gingival tissue did not significantly differ.

Moreover, in our study, the PI and GI indices, which represent the degree of gingival inflammation, were not significantly increased during the orthodontic treatment, i.e. before, during, and after complete orthodontic canine movement period. Therefore, it is unlikely that gingival inflammation will play a major role in the cyclical changes in HA levels. We instead suggest that the tissue remodeling process from both soft and hard periodontal tissue by orthodontic force create the cyclical changes in HA levels observed in this study.

However, because of the considerable variations in HA levels throughout the study and the lack of significant association found between HA levels and the applied force in this study, it is reasonable to conclude that HA is not a good biomarker for monitoring changes in the periodontal tissue during orthodontic tooth movement. Moreover, the findings from this study are difficult to be interpreted, since it is still unknown about the origins of HA detected in GCF and whether or not varying HA levels represent catabolic or anabolic changes in the periodontal tissue in response to the orthodontic force. HA can be synthesized and regulated by several types of cells, enzymes, and conditions, so more definitive studies regarding the localization of HA in the periodontal tissue during orthodontic tooth movement will provide important information regarding the complex nature of HA in GCF.

LIMITATION OF THIS STUDY

1. The rate of individual tooth movement is difficult to be controlled, and it greatly varies between patients, so the duration spent to collect GCF samples varied considerably, and took up to two years to finish GCF collection. Therefore, only GCF samples from 7 canines and 3 incisors could be completely collected for HA analysis, and then the sample size in this study was rather small.

2. Another concern about the quantities of GCF contents after being stored in the -80°C freezer, especially the collected GCF samples during the beginning of study, such

as at T0 or the leveling phases, was also raised. This might directly affect the true HA levels at any time, and would definitely interfere with the result interpretation. Why did we have to store the GCF samples in the freezer until we finished collection of GCF in each patient? These were because there were variations in HA levels in the normal population or even within the same person at different times of GCF collection, and there were variations between each ELISA run. Therefore, it is imperative that quantitative analysis of HA by ELISA in all GCF samples from one patient be performed in the same run to avoid such variations as much as possible.

3. There were several factors that affected HA levels in GCF besides the degree of inflammation in periodontal tissues. These included the presence of hyaluronidase enzymes in the gingival crevice from host or bacterial cells, great variation in HA levels between individuals, and the method of GCF collection. Despite repeated good oral hygiene instructions for the patients in this study, inflammation always took place at the gingival tissue around the teeth, since the GI and PI scores indicative of the degree of inflammation appeared to be continually increased, even though they were not statistical significant. This might be due to the bulky appliances that made good cleaning almost impossible.

4. The data about baseline levels of HA in normal Thai population, whose ages are about the same as our patients, have not been received before the commencement of this study. Analysis of HA levels in normal population is necessary because this will give us a profile of HA changes in human GCF even without orthodontic force, which will be useful for comparisons with the profile of HA changes in an orthodontic patient.

5. Is the total protein content within the GCF samples a good internal control to normalize HA levels, or should a particular type of proteins be used as an internal control?

SUGGESTIONS FOR FURTHER STUDY

1. More frequent GCF collection should be performed in the early phases of treatment, such as at T0 and the leveling phases. However, the number of total samples in one tooth is limited by the restricted number of wells in the ELISA plate. Therefore, we cannot increase the total number of samples from one tooth beyond the number of remaining wells after subtracting some wells for the standards. Moreover, the repetition of measurements, i.e. duplicate or triplicate from one GCF sample, should be considered as well.

2. The sample size is required to be larger as well as a better control of oral hygiene for each patient.

3. Consider a particular type of proteins used as an internal control instead of total protein contents.

4. Conduct a study to determine the baseline HA levels in normal Thai population.

5. The distance and direction of tooth movement should be measured from a study model to detect any horizontal and vertical tooth movement. This will make comparisons between HA levels and the amount of tooth movement more accurately.

6. Analyses of other biomarkers or inflammatory markers, such as cytokines, are recommended to be conducted in conjunction with HA analysis. In addition, other relevant studies about the effects of different magnitudes of force, types of coil spring, and main arch wire types, on changes in HA levels are necessary to determine any correlation between changes in HA levels and orthodontic tooth movement.