CHAPTER V
DISCUSSION

C-6-S levels in human GCF as a biomarker for alveolar bone resorption during orthodontic tooth movement

In this study, the C-6-S was detected in human GCF samples collected from teeth undergoing orthodontic tooth movement. This was the first report to show the detectable C-6-S levels in GCF collected from teeth undergoing tooth movement by using a quantitative technique, i.e. ELISA, and our newly synthesized WF6 monoclonal antibody. Consistent with our result, the C-6-S levels in dogs' GCF could be detected by using the ELISA technique and the 3B3 monoclonal antibody (Shibutani *et al.*, 1993). However, the dogs' GCF samples were not collected from teeth undergoing tooth movement like in our study. Several previous studies used an electrophoresis method and the densitometer to semi-quantitatively analyze the amounts of chondroitin sulfate from human GCF (Last *et al.*, 1988; Samuels *et al.*, 1993; Pender *et al.*, 1994; Baldwin *et al.*, 1999). However, none of these studies could distinguish the specific form of chondroitin sulfate, i.e. C-6-S from the C-4-S, which is more abundantly found in human alveolar bone. In addition, the electrophoresis method is literally not a quantitative method and cumbersome, and also requires a lot of sample manipulations, so it is not appropriate to use this method to analyze a large number of samples at a time and to be developed as a quick chair-side diagnostic tool. Consequently, with the use of ELISA technique and our newly synthesized monoclonal antibody WF6, it is now feasible for monitoring changes in the deep periodontal tissue during orthodontic tooth movement from a large number of samples. Moreover, it is suggested by the results from this study that the ELISA technique and monoclonal antibody WF6 be developed for clinical uses as a quick chair-side diagnostic tool.

The source of chondroitin sulfate in GCF is still unclear, since chondroitin sulfate comprises approximately 17% of total GAGs in gingiva (Bartold, 1987) and only

a minor component in periodontal ligament (Pearson and Gibson, 1982), but is present in much higher amounts in mineralized tissue, i.e. human alveolar bone and cementum (94% and 60% of total GAGs, respectively) (Waddington *et al.*, 1989 and Bartold *et al.*, 1988, respectively). According to the relative bulk and high content of chondroitin sulfate in human alveolar bone, it can therefore be assumed that the alveolar bone is a main source of chondroitin sulfate detected in GCF. In addition, the molecular mass and amino acid compositions of chondroitin sulfate found in GCF are similar to those of human alveolar bone (Waddington *et al.*, 1994), confirming the main source of chondroitin sulfate in the alveolar bone. Chondroitin sulfate in the tissue can be found in two forms, i.e. C-4-S and C-6-S (Okazaki *et al.*, 1993). The C-4-S is predominantly found in alveolar bone (Waddington *et al.*, 1989), while the C-6-S can also be found in alveolar bone, but in less amounts (Waddington and Embery, 1991). Therefore, it is likely that the C-6-S detected in human GCF from our study is derived from the degradative process of extracellular matrix of alveolar bone that is increased during orthodontic tooth movement. This is also consistent with the hypothesis proposed by Davidovitch (1995).

Since the main component of GAGs in alveolar bone is C-4-S, its presence in human GCF has been used to predict metabolic changes occurring in alveolar bone from periodontal diseases (Last *et al.*, 1985; Embery and Last, 1989; Smith *et al.*, 1995; Waddington *et al.*, 1998; Fuhua *et al.*, 2000) and orthodontic tooth movement (Last *et al.*, 1988; Samuels *et al.*, 1993; Pender *et al.*, 1994; Baldwin *et al.*, 1999). All of these studies suggested that the C-4-S in human GCF was a "biomarker" for alveolar bone resorption. However, the findings from one study had demonstrated that the localization of C-6-S in periodontal ligament was also found to be associated with experimental tooth movement (Kagayama *et al.*, 1996). Consequently, the correlation of WF6 epitope levels of C-6-S with orthodontic tooth movement found in our study was consistent with the association found in the study mentioned above, and we therefore suggest that C-6-S is another "biomarker" for alveolar bone resorption like C-4-S.

The monoclonal antibody 3B3 detects a C-6-S epitope in serum that differs from that detected by the monoclonal antibody WF6. The results from a previous study

(Peansukmanee *et al.*, 2003) suggest that the WF6 epitope of C-6-S can be used as a cartilage destruction marker in the diagnosis of degenerative joint disease. This is in parallel with the correlation of WF6 epitope levels of C-6-S with orthodontic tooth movement in our study. Therefore, this confirms an essential role of C-6-S as a biomarker for tissue destruction, particularly in mineralized tissue like bone.

A cyclical pattern of C-6-S levels in human GCF collected from teeth undergoing orthodontic tooth movement

The cyclical pattern of changes in the C-6-S levels during canine movement with a 3- to 5-week interval between two highest neighboring values was observed in this study. This pattern appears to be corresponding with the bone cycle, which lasts for 3-5 weeks and consists of resorptive, resting, and reversal or formative phases as described by Hill (1998). The peak C-6-S levels may represent the resorptive phase of the bone cycle during orthodontic tooth movement, whereas the low C-6-S levels may then represent the reversal or formative phase, in which the osteoclastic activities are arrested (Hill, 1998). The duration of 3-5 weeks may be relevant to the appointment time for an orthodontic patient to return to the clinic for having the appliances adjusted. Besides the main reason for patient's convenience, it is possible that the C-6-S levels are highest at the fourth week during the canine movement phase, implying the maximum rate of bone resorption at that time. The maximum rate of bone resorption should be beneficial for maximum tooth movement at that time, i.e. the tooth can move more quickly due to less obstruction from bone, if the force is applied again.

In addition, the conflicting findings regarding the changes in chondroitin sulfate levels during orthodontic movement from previous studies can be explained by our results that showed the cyclical pattern of changes in the C-6-S levels i.e. rises and falls in the C-6-S levels, throughout our longitudinal study. This was because all of previous studies were designed as a cross-sectional study (Last *et al.*, 1988; Samuels *et al.*, 1993; Pender *et al.*, 1994; Baldwin *et al.*, 1999). It is possible that the low chondroitin sulfate levels detected in GCF and the non-significant changes in its levels in one study (Pender *et al.*, 1994) are due to collecting GCF samples during the formative phase or

low chondrointin sulfate levels in GCF. On the other hand, the high chondroitin sulfate levels and the significant changes in its levels in other studies (Last *et al.*, 1988; Samuels *et al.*, 1993; Baldwin *et al.*, 1999) are due to collecting GCF samples during the resorptive phase or high chondroitin sulfate levels in GCF.

To determine the significant differences between each treatment phase, the WF6 epitope levels of C-6-S in the first four weeks of the canine movement phase (M0, M1, M2, M3, and M4) and of the complete canine movement phase (S0, S1, S2, S3, and S4) were selected for comparisons. The first four weeks might represent the first alveolar bone cycle after the initial force was applied to distalize the canines during the canine movement phase or to tie a canine with a second premolar during the complete canine movement phase. The magnitude of force would then gradually decrease along with the time. At M4 or four weeks after the initial applied force, the coil springs were re-adjusted in order to maintain the same force magnitude. It was possible that this second applied force would interfere with the bone cycle, so the cyclical pattern of changes in C-6-S levels might be disturbed and could not then be predicted. With this plausible reason, after the M4 and S4, the pattern of longitudinal changes in C-6-S levels was neither predictable nor corresponded with the periods of force adjustment given onto the canines. This was one of the confounding factors which could not be controlled in this study.

Since the force was applied directly to the canines, the resorptive phase of the alveolar bone cycle only took place around the canines, leading to the degradation of extracellular matrix of alveolar bone and the release of the C-6-S into GCF around the canines. Consequently, the levels of C-6-S continually increased in the canines from M0 to M4, but not in the control incisors. The only significant difference in the C-6-S levels was found between M0 and M4 in the canine. The C-6-S levels in the incisors remained low not only from M0 to M4 but also throughout the study. This was because the force was not directly applied to the incisors. However, in this study, teeth were linked together with trans-septal fibers in order to stabilize teeth against separating forces. This stabilization was achieved by maintaining the contacts between the neighboring teeth

(Southard *et al.*, 1992). Therefore, the applied force exerted on the canine might be transferred to the incisor via these trans-septal fibers. Therefore, the periodontal tissue around the incisor might indirectly respond to the force directly applied to the canine by modest degradation of extracellular matrix and eventually release detectable amounts of C-6-S into the GCF of the incisor.

During the first four weeks after the complete canine movement or S phase (S0, S1, S2, S3, and S4), the C-6-S levels in GCF of both canines and incisors had dramatically dropped down when compared to the high C-6-S levels in the canines during the movement phase. During the S phase, the force was removed from the canines, so the degradation of extracellular matrix of alveolar bone around both canines and incisors was decreased, resulting in much lower C-6-S levels detected in this phase. It was interesting to note that the only significant difference found in this phase was between the C-6-S levels of canines and those of incisors at S4. The reason underlying this difference was still unclear. In conclusion, we have shown the detectable C-6-S levels in human GCF samples collected from both canines and incisors and the cyclical pattern of changes in the C-6-S levels in the canines from this longitudinal study. Moreover, the correlation between the high C-6-S levels, or the resorptive phase of bone cycle, and the orthodontic force in the canines has been found. It is suggested by the results from this study that the C-6-S be a novel "biomarker" of alveolar bone resorption during orthodontic movement.

Limitation of the study

As a result of the limited financial support, a small sample size and the only one marker i.e. resorption marker, were use in this study.

Suggestions for further study

In the further study, the sample size should be increase and use other biomarkers, such as cytokines, prostaglandins, etc., for better understanding of biological mechanisms of alveolar bone remodeling during orthodontic tooth movement. Furthermore, another monoclonal antibody against C-6-S, i.e. mAb 3B3

should be use simultaneously with monoclonal antibody WF6 to analyze the C-6-S levels in GCF in order to obtain a better view of changes in the bone metabolism