CHAPTER V

DISCUSSION

CS is the main component of glycosminoglycans (GAGs) in alveolar bone. The levels of CS in human gingival crevicular fluid (GCF) represent a marker for active alveolar bone and periodontal ligament turnover and have been used to investigate alveolar bone remodeling as a result of periodontal disease and orthodontic tooth movement.[13,57-58] A previous study suggested that the CS component in GCF was associated with some clinical conditions, such as untreated chronic periodontitis, healing after periodontal surgery, trauma from occlusion, and orthodontic tooth movement in which degradation of alveolar bone and periodontal ligament occurs.[59] For dental implants, CS levels in peri-implant crevicular fluid were also used for monitoring bone resorption and health status of dental implant.[44, 60-62]

Previous studies have demonstrated that CS (WF6 epitope) can be detected in both GCF and PMICF by using the ELISA method with monoclonal antibody WF6.[13, 15] It has been suggested that the concentration of CS (WF6 epitope) in GCF might provide a means for monitoring bone resorption during orthodontic canine movement and that the CS (WF6 epitope) levels in PMICF might be associated with bone resorption around miniscrew implants.

In this study, the CS (WF6 epitope) levels in GCF and PMICF were investigated in a manner similar to those used for GCF and PMICF in previous

studies.[13,15] CS (WF6 epitope) was detected in human GCF and PMICF samples collected around experimental molars undergoing orthodontic intrusion, from control molars as well as from miniscrew implants. The results showed that the median CS (WF6 epitope) levels during the loaded period (12 weeks) around the intruded experimental molars was significantly greater than that during the unloaded period (2 weeks) ($P < .05$). On the other hand, the median CS (WF6 epitope) levels during the unloaded (2 weeks) and the loaded period (12 weeks) around the right mandibular first molars and around the right maxillary second molars, which served as the controls, were not significantly different. These findings coincided with those of Samuel *et al.*[50] and of Baldwin *et al.,*[48] which suggested that the vertical component of tooth movement produced an increase in CS levels. However, those previous studies quantified the CS levels in GCF of orthodontically moved canines by using electrophoresis.[48,50] This method is a lengthy procedure and requires manipulations of the sample. Therefore, it is not suitable for a quickly chair-side method for GAG quantification. They also resemble those of Last *et al*.[30] and of Kagayama *et al.,*[29] which were an increase of CS levels in GCF at the compression side of teeth during active orthodontic movement. In addition, these findings also correspond with those of the study of Jaito *et al*.,[13] which reported that there was an increase of CS (WF6 epitope) levels in GCF around canines undergoing orthodontic movement, and that the CS (WF6 epitope) levels in GCF around incisors, which served as control teeth, were not increased. The increase in CS (WF6 epitope) in this present study may explain as follow; since the apical third of the root is the zone of main pressure in intrusion movement, mechanical stress may alter blood flow and trigger cellular degeneration.[63] Connective tissue breakdown causes a release of GAGs into GCF.[64]

CS comprised approximately 17% in gingival, 60% in human cementum, 94% in human alveolar bone and minor amount in PDL. The relatively bulk and high concentration of CS in human alveolar bone, suggests this tissue may be the main source of CS in GCF. Thus, perturbation of alveolar bone by orthodontic tooth movement would be expected to enhance the amount of CS found in GCF.[50] Therefore, it is likely that the significant increase in CS (WF6 epitope) levels in GCF around the intruded experimental molars in this study results from the degradative process of extracellular matrix of alveolar bone during application of an intrusion force. However, root resorption should be simultaneously monitored.

For a better understanding of bone resorption around the orthodontically moved teeth, the median CS (WF6 epitope) levels around the experimental molars during the unloaded period (2 weeks) were compared with those during each one-week interval of the loaded period. The results showed no statistically significant difference between the unloaded period (2 weeks) and each one-week interval of the loaded period (12 weeks) around experimental molars, control molars or miniscrew implants. This may be due to the small sample size when the data were separated into each one-week interval of the loaded period (n = 18 in the experimental group; n = 9 in the molar control and miniscrew implant groups). The loaded period was divided into sub-groups of two-, three-, four- or six-week intervals to see if there was a significant difference between the unloaded period and the subgroups. Statistically significant differences were found among the medians of CS (WF6 epitope) levels during the unloaded period (2 weeks) and those during each two-, three-, four- or six-week interval of the loaded period (12 weeks) around the experimental molars ($P < .05$). On the other hand, no statistically significant difference was found around

control molar groups and around miniscrew implants groups. This finding may suggest that the monoclonal antibody WF6, a novel product of Thailand Excellent Center for Tissue Engineering, Faculty of Medicine, Chiang Mai University, can detect CS (WF6 epitope) in GCF and in PMICF. CS (WF6 epitope) may serve as a biocmarker of alveolar bone turnover within the first two-week interval of orthodontic molar intrusion. An increased sample size in future investigations may reveal the association between the CS (WF6 epitope) levels and the orthodontic loading.

The CS (WF6 epitope) levels in GCF of maxillary right second molars were greater than those around mandibular right first molars. The explanation may be that the maxillary second molars may affect from the transceptal fiber and there is pooling of sample fluid from the nearby intruded experimental molars to the gingival sulcus of the maxillary right second molar. So, the use of the mandibular first molar is suggested as a control tooth to avoid these effects. This suggestion is supported by the finding of Sari and Uçar,[14] who assessed interleukin-1β in the GCF of orthodontically moved maxillary canines and used mandibular canines as controls.

The results of this study may emphasize the role of CS (WF6 epitope) level as a biomarker for alveolar bone resorption around orthodontically moved teeth and also around miniscrew implants, and may be used as a chair-side diagnostic tool during clinical orthodontic practice in the future. These results should be interpreted carefully because a small sample size was used. Our suggestions for further study are as follows:

- It is of interest to monitor CS (WF6 epitope) in the first four-week interval thoroughly.
- The sample size should be increased if possible.

- Other biomarkers, especially alveolar bone resorption markers and root resorption markers, should be simultaneously monitored for better understanding of biological mechanisms of alveolar bone remodeling during orthodontic tooth movement.
- Periodontal status should be monitored.

Limitations of the study

1. As a result of specific criteria for the volunteers, a rather small sample size was used.
2. As a result of the limited financial support, only one biomarker was used in this present study.