CHAPTER V

DISCUSSION

Analysis of gingival crevicular fluid has been used to study orthodontic tooth movement because it was non-invasive and provided ease of repetitive sampling. Remodeling of alveolar bone and periodontal ligament induced production of various cell mediators and enzymes that could be detected as biomarkers of orthodontic treatment.[51] Alkaline phosphatase was considered to be a marker of bone deposition, which was a part of bone remodeling during orthodontic tooth movement.[52]

During orthodontic intrusion, periodontal ligament fibers were stretched and bone formation was found along the major part of the alveolus. Only apical fibers were compressed.[53] The stretching of the fibers may induce a bending of the alveolar wall, and may cause new bone formation to harmonize the orthodontic and orthopaedic perception of the periodontium under loading.[54] In addition, intruding movement may cause formation of new bone spicules in the marginal region due to the stretch of the principle fibers.[55]

The present study found that alkaline phosphatase levels in gingival crevicular fluid around experimental molars were significantly increased under orthodontic intrusion. The present results were not similar to those of Isik *et al*., the only article investigating orthodontic intrusion, that showed the decrease of bone alkaline phosphatase with force application.[18] This discordant data may involve the

experimental duration. The experiment of Isik *et al*. took only four weeks.[18] Such period of time might be too short to discover the raise of alkaline phosphatase level which reflected the bone formation. On the other hand, the intruding force was applied for twelve weeks in our study. The duration of this present study might be sufficient to reveal the significant increase of alkaline phosphatase level in gingival crevicular fluid as bone was remodeling.

Other studies, which monitored alkaline phosphatase levels, were carried out with non-axial tooth movement.[6-9] The levels of alkaline phosphatase from tension sides; however, were significantly increased in comparison with control teeth or even compression sides of moving teeth. These data was in accordance with our present study in which tension area was primarily presented in periodontium of intruding teeth.

The increase of alkaline phosphatase around orthodontically intruded molars might be explained by different tissue reactions presented in various phases of tooth movement.

Several studies showed early elevation of alkaline phosphatase activity after initial orthodontic loading, although little or no tooth movement occurred.[6, 8-9] These trends may be related to local biologic responses.[8] The early stage of orthodontic tooth movement involves an acute inflammatory process. Alkaline phosphatase was increased with inflammation.[8, 32, 56] Thus, early inflammatory reaction, that was known to occur during early stage of orthodontic tooth movement, might be found with an increase of alkaline phosphatase activity.[8] However, the elevation of the enzyme was also significant as gingival index values increased.[33, 39] This phenomenon

was related to host defense mechanism. Alkaline phosphatase, produced by polymorphonuclear cells, was thought to play a host defensive role in superoxide generation.[57] For further investigation, it should be considered to control the dental plaque-induced gingivitis, which might affect the level of alkaline phosphatase. Alkaline phosphatase detection kit, which is suggested to use in further study, should be specified the bone specific alkaline phosphatase to ensure the expression of this enzyme associated with orthodontic tooth movement.

In the later phase of tooth movement, bone apposition had taken place at the tension side which mainly occurred along the alveolus.[53] The bone surface was covered with alkaline phosphatase positive osteoblastic cells.[58] Mechanical stimulation activated cells in periodontal ligament and alveolar bone. The initial event in the appositional phase consisted of chemo-attraction of osteoblasts or their precursors to the bone formation sites.[51] Precursors in the periodontal ligament cells were stimulated to differentiate into osteoblasts by activated osteocytes.[59] Subsequently, the osteoblasts continued to form osteoid matrix and mineralize through the roles of alkaline phosphatase.[51]

Orthodontic intrusion produced compression area at the root apex.[53] Interestingly, osteoblasts had roles in resorption side. At the end of bone resorption, the start of bone formation occurred.[51] Osteoblasts laid at the bottom of resorptive cavity, then formed osteoid matrix and mineralized until the defect was filled.[60] So alkaline phosphatase was likely presented in the resorption side.

In the control groups, right mandibular first molars and right maxillary second molars, there was no significant change when comparison between the unloaded and

the entire loaded period was done. The significant changes could be; however, found in some intervals of the loaded period. Nevertheless the right maxillary second molars might be influenced by experimental first molars, the neighboring teeth, due to tension force produced by supercrestal fiber and pooling of gingival crevicular fluid from the intruding teeth. Hence mandibular first permanent molars should be recommended to be control teeth for such study. This might be similar to other studies which use contralateral or antagonist teeth as controls.[6, 8]

Alkaline phosphatase could be detected in crevicular fluid around miniscrew implants in the present study. Miniscrew implants were immobile throughout this present study with the significant change of alkaline phosphatase levels in peri-miniscrew implant crevicular fluid in certain intervals during the loaded period. When comparison between the unloaded and the entire loaded period was done; however, the significant alteration was not found. Until now, no literature has been shown whether alkaline phosphatase levels in peri-miniscrew implant fluid were increased or decreased after loading miniscrew implant and using it as orthodontic anchorage. Sari and çatal revealed that the miniscrew implant, as orthodontic anchorage, did not demonstrate an increased level of IL-1β, an inflammatory mediator, during canine retraction. The authors also indicated that mechanical stress on healthy miniscrew implants may not affect the levels of IL-1β in peri-miniscrew implant crevicular fluid.[14] Intachai *et al*. showed that the chondroitin sulphate (WF6 epitope) levels, around 'immobile' miniscrew implants, between the unloaded and loaded periods were not significantly different and also indicated the significant increase of chondroitin sulfate (WF6 epitope) level in peri-miniscrew implant crevicular fluid around one failed miniscrew implant.[61] These might be in conformity

with our study that the crevicular fluid around stable miniscrew implants did not noticeably show a level alteration of alkaline phosphatase, which may be a marker for inflammation of peri-miniscrew implant tissue.[12]

Limitation of this study involved a small sample size. From the present study, the significant increase of alkaline phosphatase levels in gingival crevicular fluid around experimental intruded molars could be shown after prolonged loaded period. Thus sample size should be increased in further study. Moreover, periodontal health should be considered because it related to the level of alkaline phosphatase.[33, 39] Since another limitation of this study involved various origins of alkaline phosphatase, the detection kit should be able to measure the level of bone-specific type instead of total alkaline phosphatase for further investigation. In addition, several other biomarkers in gingival crevicular fluid should also be investigated during orthodontic intrusion, because there are a number of signaling molecules associated with tissue reactions to orthodontic force. To substantiate the presence of alkaline phosphatase in periodontium of orthodontically intruded teeth, histochemical investigation should also be performed.[62] This might be; however, impossible to study in human due to ethical aspect. So, animal study may be considered.