CHAPTER II
LITERATURE REVIEW

The review was divided into five parts as follows:

2.1 Orthodontic anchorage and its control

2.2 Development of temporary anchorage devices (TADs) especially the miniscrew implant as an orthodontic anchorage

2.3 Stability assessments of the miniscrew implant

2.4 Gingival crevicular fluid (GCF), Peri-implant crevicular fluid (PICF) and Peri-miniscrew implant crevicular fluid (PMICF)

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2.1 Orthodontic anchorage and its control

In orthodontics, the term anchorage is defined as "resistance to undesirable tooth movement". Forces that move teeth are generated by active components, such as a spring, an arch wire, or an elastomeric chain. These active components produce a force in one direction, and another force of equal magnitude in an opposite direction. This is explained by a Newton's third law of motion -"Every action has an equal and opposite reaction". Undesirable movement of anchorage unit is referred as an anchorage loss. Successful orthodontic treatment depends on controlling such undesirable tooth movement. Orthodontists have used various procedures and devices to increase anchorage value of traditional anchorages. These include incorporation of many teeth in the anchorage unit, intra-oral devices, such as a palatal or a lingual arch, Class II and Class III elastic tractions, a Nance appliance, a utility arch, a lip bumper,[1] and an anchorage bend.[67] In some conditions, extra-oral device, such as headgear, are needed as well as intra-oral anchorage to enhance anchorage situation. However, the limitation of intra-oral anchorage is movement of the anchorage teeth under orthodontic forces. The extra-oral anchorage also has limitations, because it requires

excellent patient's compliance.[1] Recently, the temporary anchorage devices have been introduced to overcome the limitations of these traditional anchorages.

2.2 Development of temporary anchorage devices (TADs) especially the miniscrew implant as an orthodontic anchorage

Temporary anchorage devices are devices that are temporarily fixed to bone in order to be used as orthodontic anchorages, and are subsequently removed.[68] The temporary anchorage devices have been developed via two lines. First category originated as osseointegrated dental implants, which provided firm osseous anchorage for orthodontic treatment. The palatal implant, palatal onplant[68] and retromolar implant[69] were included in the first category. The limitations of these devices, to be used as orthodontic anchorage, were limited space for placement, high cost and long waiting time required for osseointegration.[70]

The second category was modified from surgical mini-implants[2] or surgical fixation screw.[68] The first clinical report in using a surgical vitallium bone screw as a temporary anchorage device appeared in 1983 when Creekmore and Eklund[2] treated a patient with deep impinging overbite by inserting the surgical mini-implant below nasal cavity. Later in 1997, Kanomi[3] described a mini-implant specifically designed for orthodontic use. Then, Costa et al[4] described a miniscrew implant with special bracket-like head that could be used for either a direct or an indirect anchorage. The miniscrew implant can be placed in several locations in order to be used as orthodontic anchorage with intention not for osseointegration, but for mechanical retention. The miniscrew implant offers many advantages, such as its small size to place in any area of alveolar bone (retromolar, palatal, buccal plate, symphysis), ease of placement and removal, independence of patient's compliance, shortening treatment time, and ability to withstand immediate force loading with an adequate anchorage support.[28,68,70]

Commercially available miniscrew implant was designed in various diameters and lengths. The miniscrew implant diameter ranged from 1. 2 to 2. 0 mm, and the length from 4. 0 to 17. 0 mm[15,20,22,71-73] Furthermore, there were several miniscrew implant shapes and head types for various orthodontic application, such as taper shape

for tighter initial placement, original cylindrical shape, button-shaped head, and hole type for ligature wire and elastomeric ligature.[3,71]

Clinical application of the miniscrew implant included closure of extraction spaces, symmetrical incisor intrusion, correction of canted occlusal plane, alignment of dental midline, extrusion of impacted canines, molar intrusion, molar distalization and mesialization, intermaxillary anchorage and upper third molar alignment.[15] Suitable miniscrew implant insertion site should not be adjacent to important anatomical structures such as nerves, vessels, airways, and air sinuses. On tooth-bearing area, 2. 0 mm of safety clearance between the miniscrew implant and dental root was recommended.[20,74] In young patients, care should be taken not to place the miniscrew implant on area that might affect maxillofacial growth.[74]

The sites which were most frequently used for miniscrew implant insertion in the maxilla include interradicular spaces, both buccal and lingual plates, extraction spaces, and inferior surface of the anterior nasal spine. In the mandible, the most common miniscrew implant placement sites were interradicular spaces, both buccal and lingual plates, the lateral area of mentalis symphysis, and extraction spaces.[4,15] The proper size of the miniscrew implant depended on thickness of cortical plate and available space. In the maxilla, narrower diameter of the miniscrew implant could be selected, if it was placed between the roots. If the miniscrew implant stability depended on insertion into trabecular bone, longer screw was needed. If cortical bone provided enough stability, shorter miniscrew implant could be chosen.[75]

2.3 Stability assessments of the miniscrew implant

The most important consideration of the miniscrew implant application for orthodontic anchorage was miniscrew implant stability.[3,4,21,28,68,70,71] This stability was related to local bone quality and quantity, type of the miniscrew implant, and placement technique. Factors influencing miniscrew implant stability were amount of bone-miniscrew implant contact, compressive stress at the miniscrew implant-tissue interface,[35] diameter of miniscrew implant, and prevention of inflammation around peri-miniscrew implant tissue.[19]

Stability assessments of the miniscrew implant were previously based on the stability assessments of dental implant, including clinical assessments,[19-21,28-41]

histological assessments,[5,28,36,41-44] mechanical assessments.[4,36,47-52] and biochemical assessments by PICF analysis.[30,53-63] The stability assessments of the dental implant and of the miniscrew implant were described in detail as follows:

2.3.1 Clinical assessments

2.3.1.1 Assessment of clinical mobility

For the dental implant, implant mobility assessments were methods for evaluating implant stability, and provided important information for planning dental implant treatment. Implant mobility was a sign for lack of osseointegration.[35] The detection of implant mobility may be very specific but not sensitive at all, because it represented an implant loss in the late state.[32] Several techniques for assessing implant mobility have been reported. The Periotest® was used to detect low degrees of implant mobility after implantation. It was reported that the vertical measuring point on implant abutment, the handpiece angulation, and the horizontal distance of the handpiece from implant might affect Periotest® values.[30,36] The resonance frequency analysis was used to evaluate implant stability in both stability at placement and during maintenance periods.[33,36,37] Resonance frequency analysis might be used to monitor changes in stiffness and stability at the implant-tissue interface by means of a signal transducer connected to a frequency response analyzer (Osstell®; Integration Diagnostic, Göteborg, Sweden). The resonance frequency of the transducer-implant unit was calculated from the peak amplitude of the signal. The implant stability quotient (ISQ) was displayed as a number between 1 and 100. [40] Bischof et al[37] reported that resonance frequency analysis showed no significant difference between immediately loaded implants and delayed loaded implants over three months period.

Wijaya et al[39] developed an implant movement checker that based on microcontroller. It was designed to overcome limitation of the Periotest®, and it was sufficient for reproducibility and reliability measurement assessing for dental implant mobility.

Clinical mobility of the miniscrew implant is an important criterion for success or failure. The clinical mobility assessment, as reported in the reviewed studies, was performed in both human and animal investigations. Some investigations described

clinical mobility assessment methods in detail,[20-22] but some did not.[18,19,24,76-78] The clinical mobility assessment methods included the use of an orthodontic tension gauge[20] or cotton tweezers.[21,22] Liou et al[20] investigated horizontal miniscrew implant mobility under orthodontic loading. To assess the miniscrew implant mobility, the miniscrew implant head was connected to an orthodontic tension gauge with a ligature wire. The tension gauge was pulled mesially by applying 400 g of force. The horizontal mobility was recorded with a sliding caliper. The scale for horizontal mobility was as follows: '0' (no movement), '1' (≤0. 5 mm), '2' (0. 5 - 1. 0 mm), '3' (> 1. 0 mm). It was reported that the clinical mobility scale of all miniscrew implants was '0' (both immediately before force application and nine months later). Park et al[22] examined the success rate and investigated risk factors affecting the success of miniscrew implants. The mobility was checked with cotton tweezers five to eight months after placement. Mobility was classified into three groups: 'yes'(mobile), 'no'(not mobile), and 'unknown'(impossible to check because of overlying soft tissue). It was concluded that the inflammation around the miniscrew implant and clinical mobility were relative risk factors for miniscrew implant failure.

In an animal experiment, Kim et al[41] inserted miniscrew implants both with and without pre-drilled holes, and investigated miniscrew implant mobility by using the Periotest®, 12 weeks after insertion. They performed histomorphometric assessment by measuring bone-to-metal contact and total bone area within the miniscrew implant thread via image-analyzing software. They concluded that the Periotest® value, the bone-to-metal contact, and the total bone area within the miniscrew implant thread were better when no holes were drilled than when pre-drilled holes were used, and suggested that miniscrew implants could provide stable orthodontic anchorage without pre-drilled holes.

2.3.1.2 Assessment of clinical features

For the dental implant, the clinical feature assessments might notify an early sign of peri-implant attachment loss.[30] The clinical parameters included discomfort and persistent pain that may be associated with increased dental implant mobility. This was an early symptoms indicating implant failure.[32] In addition, the assessments of mucosal condition, such as the amount of plaque accumulation, bleeding on probing, probing pocket depth, redness and swelling of marginal tissue, had been

reported to be clinical signs of peri-implantitis.[19,21,29,30] The most common signs and symptoms included color changes in keratinized gingival tissue or in the oral mucosa, bleeding on probing, increased probing depth of peri-implant pockets, suppuration, peri-implant radiotransparency, progressive loss of bone height around the implant. More importantly, there was a loss of bony support around the dent alimplant subsequent to localized inflammation.[38]

Inflammation or infection was associated with greater potential for miniscrew implant failure.[16,19,21-23,72,76,78] Peri-implant infection was defined as persistent pain, swelling and growth of tissue over an exposed miniscrew implant head, when analgesics and antibiotics for relief were also required.[21,23,72,76] In the absence of prescribed medications, the reported symptoms were diagnosed as inflammation. An increased incidence of tissue proliferation was observed when miniscrew implants were placed at the interface between attached and free gingiva. This resulted in coverage of the miniscrew implant head.[24]

2.3.1.3 Radiographic assessment

For the dental implant, radiographic methods are probably the most widely used for pre-operative assessment prior to implant placement and for evaluation of osseointegration.[35] The digital subtraction image had been used to evaluate changes of bone density, and to measure marginal bone loss.[31,35] The absence of a peri-implant radiolucency on radiographs was used as a criterion for implant success,[34] and a radiolucency observed around the implant was diagnosed as an implant failure.[32] The use of radiographs was criticized because they were two-dimensional and difficult to be standardized.[35] Other factors, e.g., density and trabecular pattern of surrounding bone, were also important for determining peri-implant space.[34] Moreover assessment of radiographic peri-implant bone loss provided evidence of only past alveolar bone destruction, but not future peri-implant failure.[30]

For the miniscrew implant, Liou et al[20] investigated the positional change of miniscrew implant under orthodontic loading by assessing the superimposition of cephalometric tracings (before force application and nine months later). The superimpositions revealed that the miniscrew implants remained stationary under orthodontic loading in nine of 16 patients. However, the miniscrew implants were tipped forward significantly, by 0. 4 mm at the miniscrew implant head, and were

extruded and tipped forward in seven of 16 patients. It was concluded that the miniscrew implants were stable anchorage for orthodontic loading, but did not remain absolutely stationary throughout orthodontic loading. The miniscrew implants might have been displaced because of orthodontic loading in some patients. Tseng et al[76] used panoramic radiographs to investigate the stability of miniscrew implants, and reported that the failed miniscrew implants had locked in the bone only about 3 - 4 mm because of very thick surrounding mucosa in the anterior ramus region.

2.3.2 Histological and histomorphometric assessments

For the dental implant, the histological and histomorphometric assessments were used to evaluate the features of the implant-tissue interface and to investigate the stability of osseointegrated dental implant when used as an anchorage.[35] The histological assessments in the animal suggested that the bone at peri-implant was not affected by repetitive mechanical trauma and orthopedic force, and that there was normal trabecular pattern around the implants.[42,43] Histomorphometry had been used as a quantitative method for evaluating and analyzing the percentage of bone contact and bone contact area from ground sections of implants by a light microscope equipped with the computer. Melsen et al[44] evaluated histologic and histometric features of tissue reactions around dental implants, and concluded that loading forces significantly influenced both the turnover and the density of the alveolar bone in adjacent to the implants. Furthermore, the primary stability of dental implant was analyzed by histomorphometric evaluation together with resonance frequency analysis and Periotest® measurements.[36]

For the miniscrew implant, histological and histomorphometric methods have been used as quantitative assessments for establishing the percentage of bone contact and bone contact area from ground sections of miniscrew implants.[35] A light microscope equipped with a computer morphometry programme in a digital image analysis work station has been used to evaluate the percentage of bone contact to the miniscrew implant,[28,41,79,80] the degree of osseointegration after immediate loading,[41,77] and bone volume.[80]

Ohmae et al[28] determined the anchorage potential of the miniscrew implant for orthodontic loading by using clinical, histological, and histomorphometric

assessments. The results revealed that all loaded and unloaded miniscrew implants remained stable without any mobility or displacement. The histological investigation of the peri-implant condition suggested that both loaded and unloaded implants showed partial osseointegration. These findings showed that a lower amount of osseointegration did not reflect negatively on the miniscrew implant for orthodontic anchorage. It was also suggested that the miniscrew implant could be used as a temporary implant for orthodontic anchorage.

Deguchi et al[80] investigated the differences in the percentages of bone-implant contact, bone volume, and bone formation rates in the maxilla and mandible during various healing periods. The results demonstrated that mandibular implants had significantly more bone-implant contact than maxillary implants. Within each arch, the significant histomorphometric indices (found in the 'three-week unloaded' healing group) were increased fluorochrome labeling incidence, higher woven-to-lamellar-bone ratio, and increased osseous contact. In a histomorphometric and mechanical analysis, other investigators showed that the drill-free technique could offer better stability under orthodontic loading than when drills were used.[41] Heidimann et al[79] supported the view that screw/bone contact with drill-free screws was superior to that of self-tapping screws.

Freire et al[45] evaluated the bone response to statically loaded miniscrew implants of 2 lengths activated after different healing period in beagle dogs. The results indicated that bone to miniscrew implant contact values were not significantly different between the experimental and the control groups that remain 12 weeks in vivo. The low-intensity immediate or early loads did not affect stability of the miniscrew implant.

Miniscrew implant stability and the degree of screw/bone contact depended on the difference in healing times after miniscrew implant placement, the site of miniscrew implant placement, the necessity for anchorage, the type of bone, and the technique of miniscrew implant placement.[41,79-81]

2.3.3 Biomechanical assessments

The mechanical retention of the miniscrew implant should be sufficient to sustain immediate orthodontic loading. The essential factors affecting implant

stability are local bone quality and quantity, type of miniscrew implant, and the miniscrew implant placement technique.[35,82]

The stability of the miniscrew implant is used to predict mechanical retention because the histological studies performed in animals have shown that the degree of osseointegration of miniscrew implants was less than half the osseointegration of dental implants.[28,80] Most studies on bone/miniscrew attachment have focused on the effect of shear forces, using torsion strength tests or pullout tests.[15,16,18,27,81-83]

2.3.3.1 Torsion strength test and Flexion test

Carano et al[16] illustrated some methods for measuring mechanical resistance of the Mini-Screw-Anchorage-System such as torsion to failure testing, and bending to failure testing. Torsion to failure testing was performed by placing screws into a tapped brass block at a thread depth of 6 mm. A dial torque wrench with a recording device was rotated perpendicular to the axis of the screw in a clockwise direction. The maximal torque at failure and the site of failure were recorded. Flexion testing, or bending to failure testing, was performed by placing miniscrew implants into a tapped brass block at a thread depth of 6 mm. A dial bending arm with a recording device was able to deform the axis of the miniscrew implant. The maximal bending at failure and the site of failure were recorded. The results of this study suggested that the Mini-Screw-Anchorage-System screws had high resistance to failure, and were suitable for orthodontic use. They indicated that it was possible for a human being to apply a torsion force of more than 40 Ncm (about 4 Kg) to break the miniscrew implant.

2.3.3.2 Bending test

Carano et al[81] have used the bending test to evaluate the mechanical properties of three self-tapping miniscrew implant systems. The bending forces that were used resulted in curvature of the screw and, consequently, in deflection of its head. The bending tests were performed on a universal testing machine. The miniscrew implant was maintained horizontally with a specific fixture in the fixed crosshead of the machine. A prismatic bar, connected to the mobile crosshead, applied a vertical force, perpendicular to the long axis of the screw, at a speed of 1 mm per minute. The bending force resulted in a deflection of the head of the miniscrew implant. The results showed that the miniscrew implants had enough resistance to failure during

insertion, application and removal in orthodontics. In order to break the miniscrew implant, forces higher than 80 N were required.

2.3.3.3 Insertion / placement torque test

Carano et al[81] indicated that the tests performed for evaluating the torsion moments needed for the insertion of the miniscrew implant, after the site preparation (measurements of insertion torque), were clinically important. These tests determined the effort necessary to insert the miniscrew implant and provided information about the cut design and the drill-screw diameter ratio.[27,81] The interface characteristics between miniscrew implant and bone could be expressed in relation to the implant placement torque when tightening the miniscrew implant into the bone. It was thought that when the cortical bone was stiffer or the miniscrew implant diameter was larger, the implant placement torque required was greater and the stability of the miniscrew implant was enhanced. Conversely, when the implant placement torque was too small, the miniscrew implant was unstable because of its mobility.[27]

In an in vitro study, Wilmes et al[82] investigated the parameters affecting miniscrew implant primary stability. The torque measurement and the computed bone thickness were used to assess the influence of bone quality, implant design and the insertion modalities (pre-drilling diameter and pre-drilling depth) on the primary stability. The miniscrew implants were inserted in a segment of the ileum of country pigs. The insertion torque was measured by using a torque measuring system. The results revealed that the insertion torque of the miniscrew implant was significantly positively correlated with the bone quality (computed bone thickness). The miniscrew implants having a cylindrical shape were inferior to those having a conical shape. The relationship between the shaft diameter and total diameter was responsible for greater miniscrew implant primary stability. Finally, the larger the pre-drilling diameter was, the lower the miniscrew implant primary stability. However, the pre-drilling depth had a minor effect on miniscrew implant primary stability.

In a human study, Motoyoshi et al[51] determined an adequate placement torque for obtaining a better success rate for miniscrew implants that were screwed into the buccal alveolar bone of the posterior region as an anchor for orthodontic treatment. Miniscrew implant placement torque was recorded by a torque screw driver that was

accurate to 3% as guaranteed by the manufacturer. The mean implant placement torque ranged from 7.2 to 13.5 Ncm, depending on the implant placement position.

2.3.3. 4 Removal torque test

Removal torque is a measure of interfacial strength in shear, and it not only depends on the quality of the bond between the implant and the surrounding tissue, but it is also highly sensitive to the geometry of the miniscrew implant.[35] In an animal study, Buchter et al[18] determined the clinical outcome and removal torque value of two different titanium miniscrew implant systems activated with different load regimens. The results were as follows. Firstly, miniscrew implant failure was directly related to the tipping moment at the bone rim. Secondly, by reducing the main tipping moment under a threshold of 900 cNmm (300 cN and 3 mm lever arm), miniscrew implants could be loaded immediately without impairment of either miniscrew implant stability or miniscrew implant success rates. In a human study, the removal torque values of immediately-loaded miniscrew implants after clinical usage were used to confirm the suitability of the miniscrew implant for anchorage in three-dimensional tooth movements.[83] Titanium bone screws designed for fixation in craniofacial regions were used as orthodontic anchorage. The miniscrew implants were implanted buccally in the posterior alveolar crest as orthodontic anchorages. Upon completion of orthodontic treatment, they were removed, using a screwdriver with an attached torque gauge, under local anesthesia. The maximal torque required to loosen the miniscrew implant was registered. The mean removal torque value of miniscrew implants in the maxilla was significantly lower than that in the mandible. In addition, the removal torque values of 15-mm and 17-mm miniscrew implants were significantly higher than those of 13-mm miniscrew implants.

2.3.3.5 Pull out strength test

The pull out strength test is a standardized method of testing mechanical competency, or holding power, of miniscrew implants. It has also been widely used to investigate the influence of surface irregularities on cylindrical implants.[35,50] For pull-out testing, the miniscrew implant must be aligned with the axis of the testing machine. This ensures that no bending moment is created during the pull-out test, and that only axial pull-out strengths are recorded. Huja et al[50] concluded that the pull-out strength of miniscrew implants in bone depended on the site of insertion in both in the

maxilla and the mandible. The maxillary and mandibular anterior regions had the thinnest labial plates (about 1.3 mm), and these anterior plates had significantly different dimensions from the other locations examined. The mandibular posterior region had the greatest mean thickness of approximately 2.41 mm.

2.3.4 Finite element analysis assessment

The finite element analysis (FEA) is the biomechanical testing that had been widely used to predict the effect of stress on the implant and its surrounding bone. The analysis includes load transmission and stress distribution. The finite element analysis consists of a computer model of a material or design that was used to analyze specific results and also to simulate the interaction phenomena between implants and the surrounding tissues. [46] Tepper et al[47] conducted a three-dimensional finite element analysis to find ideal implant for highly atrophic maxilla, and to assess the optimal anchorage in compromised host bone. Olsen et al[52] also established and experimentally validated a new method for planning implant surgery, and predicted initial axial implant stability. Costa and Melsen[4] used the principles of finite element analysis to change design of the miniscrew implants to a conical shape. That design provided improved strength and mechanical stability.

Motoyoshi et al[51] investigated the biomechanical effects of miniscrew implant design (abutment and thread pitches) on stress distribution and stability by using three-dimensional finite element analysis. They concluded that the existence of the abutment was useful in decreasing stress concentration on bone, whereas effect of thread pitch was uncertain. Chen et al[84] used the finite element analysis to compare anchorage effect of palatal osseointegrated and non-osseointegrated implants, under horizontal and vertical forces. The non-osseointegrated implants showed same anchorage effect as osseointegrated implants. The stress on the non-osseointegrated implant surfaces was higher than that on the osseointegrated implant surfaces, but the stress was not high enough to result in failure of the implant. These results suggested that waiting for osseointegration might be unnecessary for an orthodontic miniscrew implant.

2.3.5 Biochemical assessments

Biochemical assessments were used to investigate the stability of dental implant. Several biochemical markers revealed the destruction and remodeling of peri-implant tissue. These assessments could predict long term success of osseointegrated dental implant. [30] Numerous investigators reported association between the stability of dental implant and the levels of extracelluar matrix components in peri-implant crevicular fluid (PICF). Potential diagnostic marker of stable and diseased peri-implant condition were neutral proteolytic enzymes,[30] collagenase,[56] protease activity,[30,53,57,85] prostaglandin E_2,[30,54] neutrophil elastase, myeloperioxidase, and β-glucurinidase, α2-macroglubulin, alkaline phosphatase,[53,55] and glycosaminoglycans.[58-62]

For the miniscrew implant, Interleukin 1β levels in peri-miniscrew implant crevicular fluid were used to determine the effect of mechanical stress on the miniscrew implants during being used as anchorages for tooth movement. The result demonstrated that the Interleukin 1β levels in peri-miniscrew implant crevicular fluid (PMICF) of healthy miniscrew implants were not increased under orthodontic forces.[63]

Several studies monitored GAGs particularly chondroitin sulfate (CS) in periodontal tissue and peri-implant tissue,[64,86-88] and concluded that the GAGs in PICF were similar to those in GCF.[58-62] The periodontal tissues had been suggested to be a source of detected GAGs.[58,89] GAGs are defined as linear polysaccharide units consisting of repeating disaccharide units, of which one is a hexosamine (ether D-glucosamine or D-galactosamine) and the other is a hexuronic acid (D-glucuronic acid, L-galacturonic acid or iduronic acid). Seven species of GAGs exist: hyaluronic acid (HA), chondroitin-4-sulfate (C-4-S), chondroitin-6-sulfate (C-6-S), dermatan sulfate (DS), heparan sulfate (HS) and keratan sulfate (KS). All except HA are invariably sulfated.[88] Increased levels of GAGs that was resulted from bone remodeling had been reported.[90] This was consistent with several findings involving teeth undergoing orthodontic treatment[86-88,91] and periodontal disease.[64,65] Therefore, GAG levels were valid biomarkers for underlying hard tissue remodeling.[38,65,86,92]

CS is the predominant GAG chain of alveolar bone proteoglycans. It consists of repetitive disaccharides formed by glucuronic acid and N-acetyl D-galactosamine

residues, with a mean of one sulfate ester per disaccharide, which is bound to carbon 4 or 6 in the N-acetyl hexosamine residue. C-4-S and C-6-S can be presented in the same proteoglycan molecule. Variations in chain size, degree of sulfation, and C-4-S/ C-6-S ratio are associated with tissue physiology, age of individual being, and pathological state. CS is mostly composed of C-4-S, C-6-S, and a minority of unsulfate (C-0-S). The ratio of C-4-S to C-6-S is greater in calcified tissue than that in uncalcified tissue. Furthermore, C-6-S isomer is also present a low amount, but increased with age.[90] In GCF of the patients undergoing orthodontic treatment, the correlation between the high C-6-S levels and the resorptive phase of bone cycle[66] or the compressive force in PDL had been found.[87]

Analysis of the PICF revealed certain early changes that demonstrated the existence of bone resorption, for instance, increased levels of CS. Beck et al[58] investigated levels of C-4-S and HA after an abutment placement at 2, 4, 6, and 8 days following 3 months for osseointegration period and the bone metabolic activity. It was concluded that the CS levels were related to bone resorption around dental implant. Last et al[60] investigated GAGs in PICF, and suggested that GAG constituents, particularly CS, was a potential marker for adverse tissue responses, markedly bone resorption. Okazaki et al[61] determined the CS in PICF by high-performance liquid chomatography. They found that the CS could be detected in all PICF samples. The predominant isomer for PICF contained unsatuarated 0-sulfated disccharide (ΔDi-0S), and contained unsatuarated 4-sulfated disccharide (ΔDi-4S), together with trace amount of unsatuarated 6-sulfated disccharide (ΔDi-6S). Smedberg et al[59] and Johasson et al[62] indicated that the levels of C-4-S in PICF could be used as an indicator for progressive healing and normal resting metabolic turnover of bone adjacent to implants. The results of these studies indicated that GAGs and CS levels in PICF could be used as biomarkers for bone resorption around dental implant.

2.4 Gingival crevicular fluid (GCF), Peri-implant crevicular fluid (PICF), and Peri-miniscrew implant crevicular fluid (PMICF)

Clinical similarity of mucosa surrounding tooth and dental implant resulted in the use of periodontal terms and criteria of clinical parameters of periodontal status for evaluating peri-implant status.[93] Clinical and radiographic parameters were

routinely used to assess dental implant during function and maintenance care period. Several extracellular matrix components in PICF were also investigated in order to assess the peri-implant status.[53,55,57,61]

GCF is a complex mixture of substance derived from serum, leukocyte, structural cell of periodontium and oral bacteria.[94] Host-derived substance in GCF include anti-bodies, cytokines, enzymes, tissue degradation products[95] and bone specific markers. Collagen telopeptide fragment and osteocalcin in GCF may reflect periodontal bone resorption. Nyako et al[96] measured the pH level in the PICF and the GCF, and reported that mean pHs of the PICF in both successful and failing implant sites were similar to those in the GCF around natural teeth. Eley et al[85] evaluated protease activities in PICF. Cathepsin levels were the highest, followed by elastase, dipeptidyl peptidase, and trypsin. Total enzymes activities and concentrations both correlated positively with Gingival Index and bone resorption. Boutros et al[53] evaluated neutrophil-derived enzymes that presented in GCF as risk markers for periodontal disease progression, and compared with these enzymes in PICF. The result indicated that these enzymes in GCF around natural teeth were good candidates, for study, as risk biomarkers for endosseous implant failure assessment.

The results of these studies confirmed that the enzymes and the inflammatory response of tissue surrounding implant and natural teeth were similar.[55,57] Therefore, the PMICF was theoretically similar to the PICF.

2.5 Detection of GAGs in GCF and PICF by Enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody (mAb)

ELISA is an immunoassay widely-used for measuring concentration of particular molecule in fluid such as serum or urine. The molecule is detected by antibodies that have been made against it; that is, for which it is the antigen. This method uses two different antibodies. The first antibodies react with the antigen which known concentrations. A fixed quantity of first antibody is attached to a series of replicate solid supports, such as plastic microtiter wells. Experiment solution containing antigen at an unknown concentration or a series of standard solutions with known concentrations of antigen are added to the wells and allowed to bind. Unbound antigen is removed by washing, and the second antibody, which is enzyme

linked or radiolabeled, is allowed to bind. The antigen serves as a bridge, so the more antigens in the experiment or standard solutions, the more enzyme-linked or radiolabeled second antibody will bind. The results from the standard are used to construct a binding curve for second antibody as a function of antigen concentration, from which quantities of antigen in the experiment solutions may be interpreted.[97]

A monoclonal antibody (mAb) 3B3 was used to recognize epitope of CS in GCF by the ELISA method, standard indirect immunoperoxidase technique and immunohistochemical assay. These studies suggested that the expression of CS was related to severity of inflammation, periodontal disease, and hylalinezed PDL.[64,87,98] By using the ELISA with mAb, trace amount of GAGs presened in GCF can be precisely quantified. Monoclonal antibody (mAb) WF6 is a novel mAb, developed against embryonic shark cartilage proteoglycans, was applied to be a biomarker for recognizing an epitope in CS chains. Two octasaccharides, unsaturated **D-C-C-C** and unsaturated **C-C-A-D**, were recognized by WF6. The abbreviations used for disaccharide units are:

A, GlcUA-GalNAc(4-O-sulfate);

C, GlcUA-GalNAc(6-O-sulfate);

D, GlcUA (2-O-sulfate)-GalNAc(6-O-sulfate)or ;

unsaturated C, 4,5-unsaturated HexUA-GalNAc(6-O-sulfate);

unsaturated D, 4,5-unsaturated HexUA(2-O-sulfate)-GalNAc(6-O-sulfate).

The strong similarity in structure of the two binding CS octasaccharides (unsaturated **D-C-C-C** and unsaturated **C-C-A-D**) provided a possible explanation for their similar affinity for the WF6, although they differed in sequence and thus form two specific mimetopes for the antibody.[99] The earlier investigation showed that it recognized the chondroitin sulfate C (CS-C, chondroitin 6-sulfate). Further investigation using the micro-array oligosaccharides, a novel oligosaccharide sequencing technique and computer modeling showed that WF6 epitope contained **D-C-C-C** and **C-C-A-D** oligosaccharide sequences.[100] This antibody was applied to use as a biomarker for cartilage degradation both in vitro and in vivo studies. Tiengburanatam[101] described preparation of the mAb WF6. Pothachareon[102] reported that this WF6 epitope was higher in osteoarthritis patients than in normal serum, and it was also significantly higher in rheumatoid arthritis serum. It was discussed that it

might be able to reflect the degradation of cartilage without digesting with chondroitinase. Our previous study investigated the longitudinal changes of CS levels in human GCF during orthodontic canine movement by using ELISA and mAb WF6. It was reported that the CS could be precisely detected in human GCF during orthodontic tooth movement, and that the level changes of CS was cyclical.[66]