

EFFECTS OF THE ORTHODONTIC MINISCREW GEOMETRIC DESIGN ON THE BONE
MICRODAMAGE AND PRIMARY STABILITY



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This study investigated the effects of the pitch (P) and thread shape on orthodontic miniscrew cortical bone microdamage. The relationship between microdamage and primary stability was examined. Ti6Al4V orthodontic miniscrews had custom-made thread heights (H) and pitch sizes and were classified into the following groups: $H_C P_C$ (control); $H_C P_N$ (with a narrower pitch); and $H_T P_C$ (with a taller thread height) ($H_C = 0.12$ mm; $H_T = 0.36$ mm; $P_C = 0.60$ mm; $P_N = 0.30$ mm). The orthodontic miniscrews were inserted in the pilot hole of prepared 1.0-mm-thick tibia cortical bone, and the maximum insertion torque (MIT) and Periotest value (PTV) were measured. After basic fuchsin staining, histological thin sections were examined using microdamage parameters, including the total crack length (TCL) and total damage area (TDA), and insertion state parameters, including the miniscrew surface length (SL) and bone compression area (BCA). The orthodontic miniscrews with taller thread height showed lower primary stability, minimal bone compression, and microdamage, while narrower thread pitch resulted in maximum bone compression and extensive bone microdamage. A wider thread pitch reduced microdamage, while decreased thread height increased bone compression, ultimately improving primary stability.

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CHAPTER I INTRODUCTION

1.1 Background and Rationale

In recent decades, the orthodontic miniscrews were generally used due to their ease of use together with their ability to help in the treatment of complex cases (1). They can be placed in most of the positions in the maxilla and mandible as a result of their small sizes (2). Though the orthodontic miniscrews have become popular to use, their failure rate was relatively high compared with the failure rate from dental implants (3, 4).

Since pitch and thread design are one of the related factors which associate with the miniscrew stability (5), the appropriate miniscrew geometric design could help enhance in miniscrew stability by decreasing bone stress and displacement of the orthodontic miniscrew (6). Seeing that orthodontic miniscrews with different geometric designs can affect the miniscrew primary stability by causing difference in pullout force (7), insertion torque, removal torque and Periotest value (8).

The study of the customized orthodontic miniscrews that were different only in their pitch showed the increasing trend of the placement torque and pullout force when the pitch was lower (9). However, both placement torque (8) and pullout force (10) are the force in the axial direction, most of the orthodontic forces are conducted in the lateral direction (11). The mechanical test in lateral direction may be needed to simulate force in the clinical situation.

The intense bone microdamage could affect orthodontic miniscrew initial stability by decreasing the bone-to-implant contact which was led to the weakening of the resistance to orthodontic immediate loading (12). It also influenced the secondary stability since the bone microdamage was related to the resorption cavity in the bone remodeling process (13). Hence, for the better understanding of the miniscrew stability with different miniscrew design, the amount of bone microdamage from each design should be clarified.

1.2 Research Question

Which thread height and pitch size cause the most primary stability and the least microdamage by using 1.6-mm MSIs in porcine cortical bone?

1.3 Research Objective

To evaluate the effect of thread height and pitch size on the primary stability and microdamage in cortical bone.

1.4 Research Hypothesis

Larger thread pitch and smaller thread height produce proper insertion torque, lower Periotest values and less amount of bone microdamage.

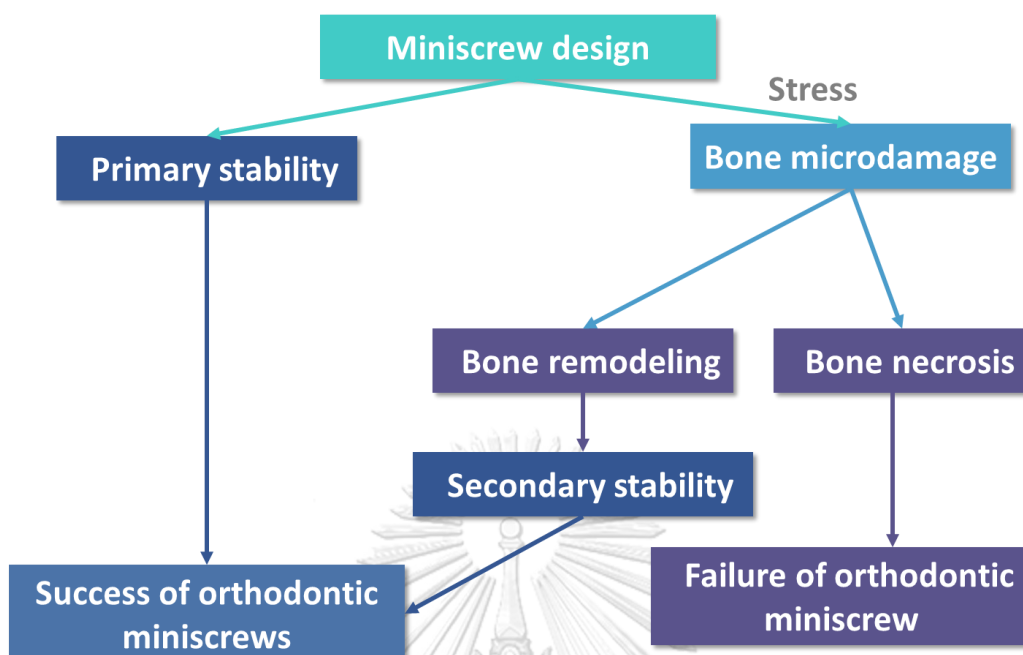
1.5 Benefits of This Study

This study will clarify the influence of the orthodontic miniscrew designs to the primary stability and the degrees of bone microdamage which can refer to the tendency of the miniscrew stability. It can help in developing of the proper miniscrew design in the future.

1.6 Keywords

Orthodontic miniscrew, Insertion torque, Bone microdamage, Cortical bone, Geometric design, Periotest, Laser confocal microscope, Thread height, Thread pitch, Bone condensation

1.7 Conceptual Framework



CHAPTER II REVIEW LITERATURE

2.1 Temporary Anchorage Devices (TADs)

Temporary anchorage devices (TADs) are biocompatible devices fixed to the bone with the purpose of facilitating tooth movement. These devices are subsequently removed after treatment (14) TADs offer several benefits beyond conventional anchorages due to their ability to provide absolute anchorage. These benefits include (15):

- No or reduced dependence on existing dentition.
- Requiring for less patient compliance.
- Application of continuous force instead of intermittent force.
- Use of mostly simple surgical techniques for insertion.
- Lower cost compared to many other alternative surgical procedures, such as orthognathic surgery.
- Immediate loading of orthodontic force as the devices rely on mechanical retention instead of osseointegration.

These armamentaria were classified into two categories based on their provenance (16). The first group originated from osseointegrated dental implants, which included retromolar implants and palatal implants. The second group derived from surgical mini-implants, such as the small screw used by Creekmore and Eklund (17) , the mini-implant specifically designed for orthodontic purposes described by Kanomi (18) , and the bracket-like head screw explained by Costa et al.(19) as shown in Figure 1.

For a comprehensive perspective, Cope (14) presented a classification depicting two major categories of temporary anchorage devices (TADs): biocompatible (Figure 1) and biologic in nature (Figure 2). Each category was further divided based on the mode of attachment to the bone: biochemical (osseointegrated) or mechanical mechanism. To illustrate, a palatal implant intended for osseointegration would fall under the biocompatible TAD category, while an orthodontic miniscrew designed for mechanical retention would also be classified as such.

The biological TADs are composed of an ankylosed tooth adhered to bone biochemically and a remarkably dilacerated tooth adhered to bone mechanically (Figure 2).

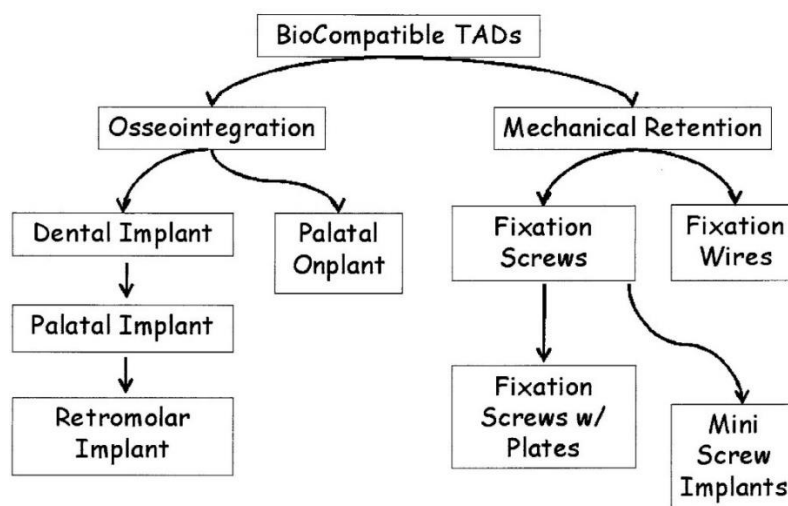


Figure 1 Biocompatible temporary anchorage devices, Classification of temporary anchorage devices.

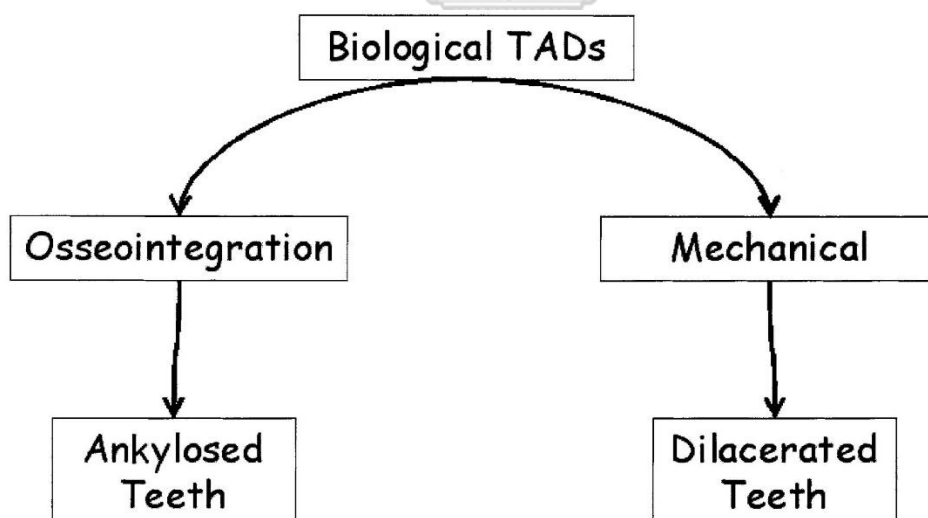


Figure 2 Biological temporary anchorage devices, Classification of temporary anchorage devices.

2.2 Orthodontic Miniscrew

In 1945, Gainsforth and Higley introduced the idea of using vitallium screws as orthodontic anchorages (20). They performed immediate loading canine retraction on six dogs, although all screws were lost within 16-31 days. However, this idea has had a significant and lasting impact on the development of orthodontic anchorage techniques, influencing the field to this day.

However, it took a considerable amount of time before vitallium screws were first employed in humans as orthodontic anchorages for deep bite patients (17). Even though this was succeeded, due to the surgical technique was not widespread acceptance, these devices were not generally used at that time.

More recently many types of temporary anchorage devices have been developed such as onplants (21), miniplates (22), palatal implants (23) but orthodontic miniscrews are now the most commonly used as absolute anchorage devices (24-27).

Most orthodontic miniscrews are made from titanium alloys (28) which are ostensibly biocompatible material allowing osseointegration between endosseous implants and bone (29). Nonetheless, when comparing titanium alloy orthodontic miniscrews with stainless steel orthodontic miniscrews, which the later ones have less biocompatibility (30), there was no significant difference in histological finding and mechanical stability between both. The conclusion is turned out to be that both titanium alloy and stainless steel orthodontic miniscrews are eligible for immediate orthodontic loading (31). Consequently, the osseointegration is not the priority for orthodontic miniscrews because the primary stability is derived greatly from mechanical stability immediately after implantation which is also the key determinant in both short-term and long-term stability (32, 33).

Normally orthodontic miniscrews are composed of three parts: head, neck, and body. For the head, there are varied in design to be used with different auxiliary devices including ligature wires or coil springs. There are two different sizes for the neck. Firstly, the small neck which is usually used in general cases. The other is the long smooth neck which is going to be used in thick mucosa cases such as palatal mucosa or retromolar area (34, 35). For the body, the orthodontic miniscrews with

conical shape have relatively lower risk for root contact than the cylindrical shape ones due to the thinner tip (34). In addition, it was found that the conical shape orthodontic miniscrews have superior primary stability than the cylindrical ones on the grounds of their greater removal torque (36). As a result, the conical shape orthodontic miniscrews are more generally used than the cylindrical shape (34). However, the conical shape orthodontic miniscrews may cause the amount of bone compression and compromise orthodontic miniscrew stability by incurring cell damage in the cortical bone (37). These bone over compression can be different depending on the placement technique which is another factor that influences the primary stability. The self-drilling technique allows orthodontic miniscrew insertion without predrilling procedure (38), while this is mandatory for the self-tapping technique. It was pointed out that insertion torque is noticeably greater for the self-drilling technique than the self-tapping technique (39). Many previous studies revealed that the success rate from self-drilling orthodontic miniscrews has exceeded that from self-tapping orthodontic miniscrews due to the higher bone-to-implant contact (39-41) and this could also facilitate bone remodeling (40). In spite of these advantages from self-drilling orthodontic miniscrews, they also generate preponderant pressure than self-tapping orthodontic miniscrews (39) leading to the more bone microdamage (42). Thereby the pilot drilling is essential in the thick cortical region to reduce the risk of the complications while this is not necessary for the thin cortical region such as maxillary bone (39).

Stress in the cortical bone around orthodontic miniscrew is also important to the treatment success. It was pointed out that the increase in the miniscrew surface help reduce in bone stress (6, 43). Seeing that the number of threads is consistent with the surface area, the less pitch value and the more thread height also increase the miniscrew surface area (6, 7).

2.3 Miniscrew Geometric Designs

Nowadays, there are variety of the orthodontic miniscrews that are commercially available for the clinician to be chosen (7, 44-46). Each of them is

different in their diameter, length, pitch, thread height, thread angle, apical face angle, lead angle and flank (

3) (7). Among all of these characteristics, Radwan and coworkers (7) found that the primary stability could be enhanced by the increase in pitch width, flank, thread angle, apical face angle and/or lead angle despite the decrease in thread-shape-factor (TSF). TSF is the ratio between thread height and thread pitch which was first introduced by Chapman et al.(47) then this value was applied to evaluate its influence to the stability of orthodontic miniscrew (44, 45). There was also a study focusing on the thread characteristics including thread pitch, thread height and the TSF and they indicated the inverted relationship between the pitch and the maximum insertion torque (44, 48, 49). Not only the maximum insertion torque but also the pullout strength that was affected by the thread pitch which was conjectured that the narrower thread pitch provides more surface area than the wider thread pitch resulting in the friction at the bone-to-implant contact (9).

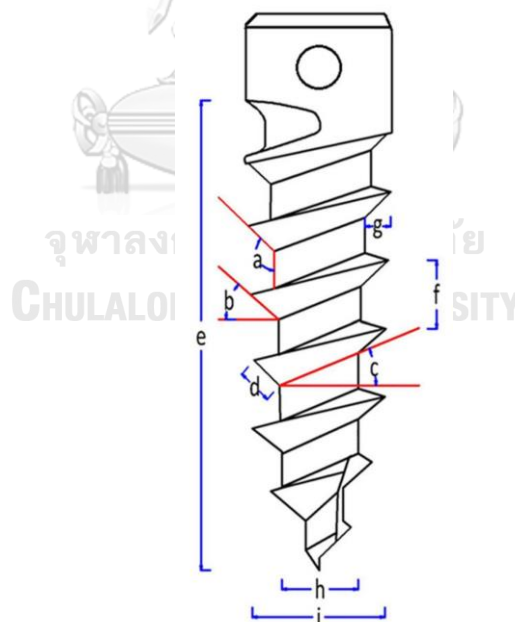


Figure 3 The miniscrew geometric designs (a, thread angle; b, apical face angle; c, lead angle; d, flank; e, length of screw; f, pitch width; g, pitch depth; h, minor diameter; i, major diameter)

With respect to the force application, the reduction in thread pitch tended to help extenuate the stress in the surrounding cortical bone (50). Moreover, the other composition of the thread like thread height was also considered. Shen and coworkers found that thread height played more important role than thread pitch in reducing of cortical bone stress while thread pitch had an influence on cancellous bone (6). In another finite element analysis about the thread design, it was documented that orthodontic miniscrew with greater thread height would generate higher maximum stress on the bone and thread elements. In addition, the displacement can be also increased when the thread height increased (10). With the same external diameter, the bigger thread height would also lead to the smaller core diameter which can compromise the miniscrew strength when the critical bending moment is transmitted to the orthodontic miniscrews (51).

2.4 Stability of Orthodontic Miniscrew

It has been delineated that orthodontic miniscrews derived their stability from two phases (52). Primary stability which is explicit immediately after insertion is regarded to be the critical key defining the success rate of orthodontic miniscrews (52, 53). Mechanical retention, which is influenced mostly by miniscrew shape, bone quality and modality of insertion, is the function of this phase of orthodontic miniscrew stability (37, 41, 54-57). Thanks to this primary stability, the immediate loading can be performed in orthodontic miniscrews as the anchorage for orthodontic tooth movement (55). Many studies supported that the thickness of cortical bone played an important role in the primary stability of the orthodontic miniscrews seeing that the stress was mainly concentrated in the cortical bone (55, 58, 59). Furthermore, many studies suggested that there is a high correlation between cortical thickness and insertion torque (52, 60-62). Even though high insertion torque indicates superior primary stability, too high insertion torque can cause mechanical damage to the adjacent tissue and also high loss rate (37, 63-65). If this damage in the cortical bone is too drastic, the stability of orthodontic miniscrews can be

exacerbated (12, 66). This primary stability is also necessary for the bone formation in the later phase which is called the secondary stability (52, 67).

The secondary stability is achieved by the osseointegration concurring with the healing period of the surrounding bone (52, 57, 68, 69). It has been presented by Ure et al. that the primary stability of the orthodontic miniscrews would be declined during the first three weeks after orthodontic miniscrew placement, then the secondary stability arisen from bone healing would help increase in overall stability (68). They also suggested that the duration of three weeks after insertion is the cut point whether the orthodontic miniscrew would fail or not (68).

There are a lot of methods applied to measure the orthodontic miniscrew stability such as insertion torque (31, 36, 37, 39, 44, 52, 61, 62, 70, 71), pullout strength (41, 44, 72), removal torque (31, 36, 39, 69), mobility test (7, 8, 40, 73), bone-to-implant contact (31, 36, 39-41, 72), radio frequency analysis (36, 37). Rather than these parameters, there are some extra factors that could compromise the orthodontic miniscrew stability which is whether it is placed in either the keratinized tissue or nonkeratinized tissue. Placement of orthodontic miniscrew in the nonkeratinized tissue has been proved to be the risk factor of inflammation and bone resorption around the orthodontic miniscrew ensuing with the miniscrew loss (55, 68, 74, 75).

The another is to place the orthodontic miniscrew contacted with dental root. It was evidenced that placement of orthodontic miniscrew contacted with dental root can disparage orthodontic miniscrew stability and aggrandize the risk of miniscrew failure (76, 77).

2.5 Periotest

Periotest is a non-invasive method used to measure miniscrew stability in lateral direction (8) which is more associated with the orthodontic force than the axial force such as insertion torque measurement or pullout test (7). Originally it was used to measure tooth mobility due to their positive correlation to the bone loss (78). It also has been used to measure dental implant stability by referring to the

bone rigidity around the implant (79). Periotest can assess the damping capacity and stiffness of the peri-implant tissue by quantifying the contact time of the electronically determined rod on the implant (80). While testing, it is important that the rod and test surface distances should be kept between 0.6-2.0 mm and perpendicular to each other. The Periotest values range from -8 to +50 which indicates from the lowest mobility to the highest mobility (78). There is some clinical limitation for Periotest to measure the implant stability in mesio-distal direction (81). However, in some experimental procedure that was conducted to measure the implant stability after insertion into the long bone can observe the Periotest values from all four directions. The mean values from three times measurement in all four directions were obtained to analyze the implant stability (82).

The success rate of orthodontic miniscrew was also evaluated by the Periotest value at the time of insertion and brought to the conclusion that the low Periotest value can help in prediction of prognosis of miniscrew placement (73). From this study the mean Periotest value in the success group is 4.8 while the failure group is 7.0 (73).

2.6 Bone Microdamage

Many factors of miniscrew placement can result in different microdamage burden. Overtightening insertion demonstrated more microdamage to the cortical bone than normal insertion (83). A higher value of the bone microdamage was also observed from the more taper in shape of orthodontic miniscrew while the miniscrew diameter seemed to have no influence on the linear damage parameter (71, 84). In 2012, Yadav et al. (42) have compared the amount of microdamage originated from different insertion techniques and sites of insertion. They concluded that there was greater bone microdamage monitored from self-drilling technique comparing with self-tapping technique. On their observation, they also detected the diffuse microdamage adjacent to the orthodontic miniscrew located up to 300 μm from the orthodontic miniscrew interface. This pattern of bone microdamage was first described as a fine network of crack in submicron level under light microscope in

fatigue-loaded human tibia bone (85). This type of bone microdamage was considered to help in bone plasticizing after fatigue loading (86). In contrast, the linear damage was considered to induce brittle fracture of the bone (86). Even though this diffuse pattern of the crack was suggested to have a role in energy dissipation preventing the crack from propagating into broader crack and finally leading to complete failure (87), the existence of this submicron-size crack in bone has been inspected in its mechanical aspect that it resulted in the subsidence of bone stiffness and toughness assimilative with the linear-pattern damage (88, 89).

Following the miniscrew insertion, the numerous amounts of microdamage generated in the supporting bone results in remarkable bone remodeling (42, 71, 83, 84, 90). This remodeling activity occurs in response to the bone microdamage in order to remove and replace them with new bone matrix for the maintenance of bone integrity (91). Unlike the diffuse microdamage, the linear-type microcrack has profound association with the resorption cavity (92). The study from Seref-ferlengez et al. (88) provide important insights of the fate of diffuse microdamage about its repair process which is not relative to any resorption cavity in the bone but there are some mechanisms accounting for direct repair of this submicron-size crack. For the spatial relation of these type of bone microdamage to the bone-to-implant interface, both of them were observed together at the interface by which the linear cracks mostly appeared behind the diffuse damage (90). In consistent with the general microdamage caused by fatigue loading, the bone microdamage caused by orthodontic miniscrew insertion also presented the bone remodeling response around the orthodontic miniscrew including the resorption cavities (90). Additionally, after being damaged by the insertion procedure, the supporting bone generally encounters with the immediate orthodontic loading (25, 93) which contributes to the additional stress to the cortical bone (58, 94, 95). The excessive damage of surrounding bone could minimize the bone-to-implant contact (12). As a consequent, the resistance to miniscrew displace could be compromised.

2.7 Detection of Bone Microdamage

The studies about bone microdamage have long been conducted mostly in the pattern of fatigue loading inducing bone microdamage and the following complications (13, 66, 85-89, 96-99). In the orthodontic field, bone microdamage has been recently investigated in the last decade (42, 71, 83, 84, 100, 101). They were conducted mostly to investigate the bone microdamage around orthodontic miniscrews. One study from Wawrzinek et al. (83) used scanning electron microscope to compare the bone linear microcrack around the orthodontic miniscrews that were tightly and normally inserted. The parameters they used were number of cracks, sum of crack lengths, the longest crack and the maximum radius of crack. A few years later Lee and Baek (71) analyzed the bone microdamage around different miniscrew types by using conventional microscope. Hematoxylin & Eosin dye was used in staining process to investigate the linear crack in the same parameters as the aforementioned study (83). The specimen preparation and staining process of these two methods can cause extra bone microdamage aside from miniscrew insertion process which will be called "the artifactual microdamage". These damages were indistinguishable from the main interesting bone microdamage. On the other hand, there are other methods that can help separate them. The meritoriousness of the en bloc staining with basic fuchsin has been proven to be able to differentiate the artifactual microdamage from the cracks caused from miniscrew insertion and even expediently when observing under fluorescent microscope (84, 91, 102, 103). This method was adopted to inspect the bone microdamage generated from miniscrew insertion in many studies (42, 84, 90). With this method, there was evidence that both diffuse and linear pattern of microdamage can be found (42, 90). This method was not only used in the field of bone microdamage from orthodontic miniscrew, but also in the field of bone microdamage from fatigue loading (86, 96, 104).

Recently, the new method in exploring bone microdamage around miniscrew has been proposed. The sequential staining technique with different fluorescent dyes distinguished the bone microdamage originated from each procedure composing of specimen preparation, miniscrew insertion and miniscrew removal (100, 101). However, finally, the bone microdamage from all procedures were assembled

together. There is another consideration method that has been used to detect bone microdamage non-destructively by staining with precipitation of BaSO_4 then imaging with micro-CT or electron-microscope in the back-scattering mode (97, 98, 105). Although this approach is the non-destructive and three-dimensional method in the bone microdamage detection, the limitation is that BaSO_4 can precipitate in all void space non-specifically including vasculature and damage spaces (97, 98). The quantitative analysis of the bone microdamage from this method was based on the image intensity measurement of the staining level (97, 98, 105).



CHAPTER III MATERIALS AND METHODS

3.1 Material

3.1.1 Porcine Tibia Bone

The bone tissues used in this study were attained from the tibia of the porcine. Even though the porcine bone's density and structure were not totally the same with the human jawbone, the reasons to use this portion were that it's uniformly flat than the jawbone. Make it was easier to make the bone in evenly cortical thickness.

The fresh porcine tibias was deperiosted and prepared into fifteen blocks with cortical bone thickness of 1.0 mm and dimension of 15 x 12 mm. The trabecular bone was excluded since it has been proven that the stress that occurred during insertion was mostly concentrated in the cortical bone and less in the cancellous bone, PDL or root (58).

3.1.2 Orthodontic Miniscrew

Fifteen titanium orthodontic miniscrews with 1.6 mm in diameter and 6 mm in length were custom manufactured into the specific dimension of thread heights (H) and pitch sizes (P) in the present study. These orthodontic miniscrews were varied into 3 groups by the differences in their thread geometry: $H_C P_N$ ($H_C=0.12$ mm, $P_N=0.30$ mm), $H_C P_C$ ($H_C=0.12$ mm, $P_C=0.60$ mm) $H_T P_C$ ($H_T=0.36$ mm, $P_C=0.60$ mm). From these designs, $H_C P_N$ and $H_C P_C$ were different only in their pitch sizes while $H_C P_N$ and $H_T P_C$ were different only in their thread heights. To confirm the geometric dimension, these orthodontic miniscrews were observed under scanning electron microscope using an accelerating voltage of 15.0 kV and current of 10 μ A (Figure 4)

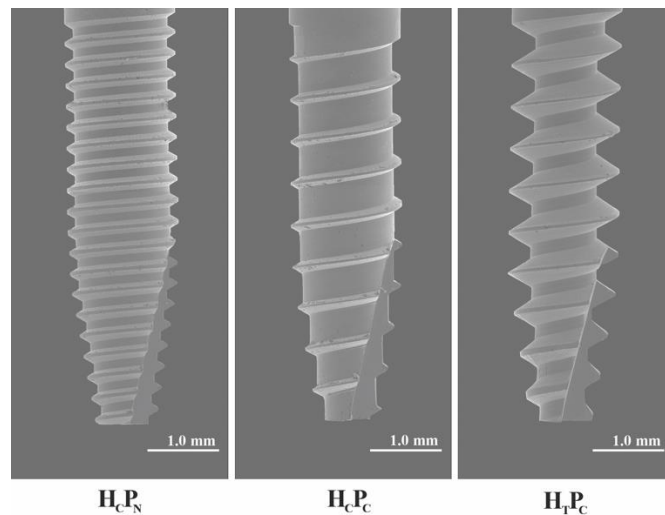


Figure 4 The custom-manufactured orthodontic miniscrews were scanned using a scanning electron microscope at 25x magnification to verify their geometric dimensions. $H_C P_N$ design: thread height (H_C) = 0.12 mm, pitch (P_N) size = 0.30 mm; $H_C P_C$ design: thread height (H_C) = 0.12 mm, pitch (P_C) size = 0.60 mm; $H_T P_C$ design: thread height (H_T) = 0.36 mm, pitch (P_C) size = 0.60 mm.

3.1.3 Pilot Drills

Cylindrical carbide burs of 0.7 mm in size were used to make pilot hole before miniscrew insertion (Saito Seisakusho, Tokyo, Japan).

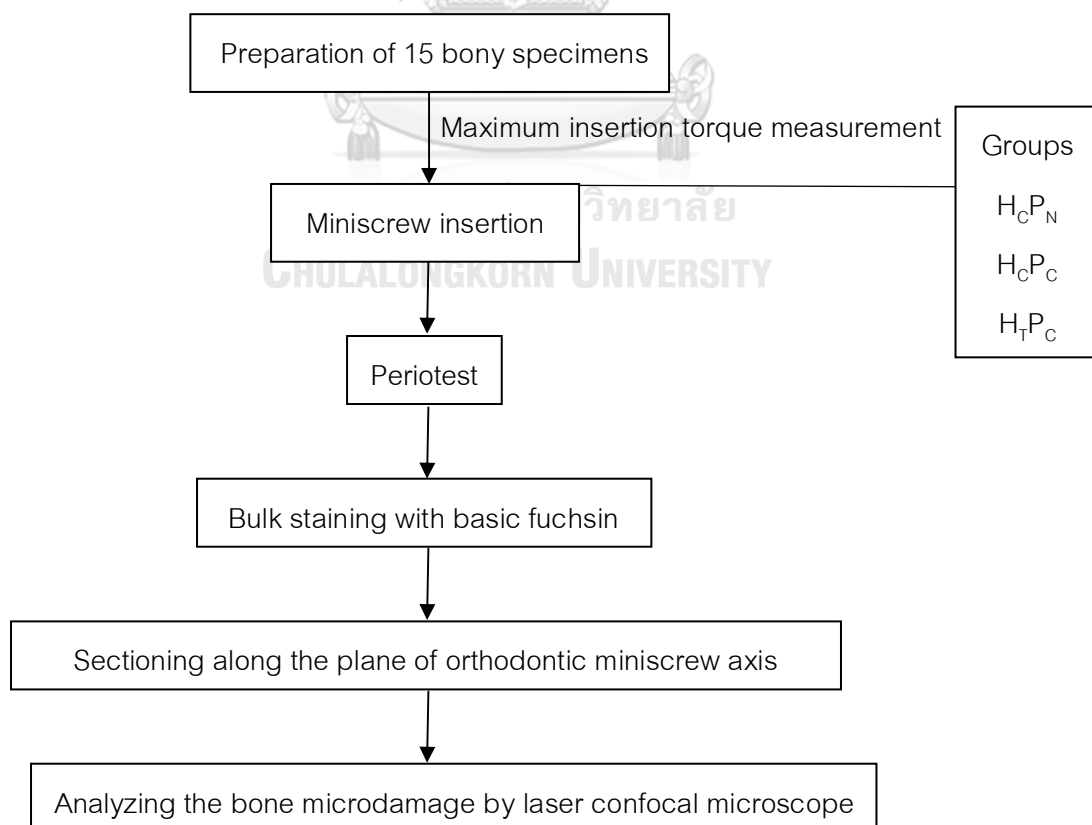
3.2 Equipment

- Scanning electron microscope (SEM; S-4500; Hitachi High-Tech, Tokyo, Japan)
- Digital torque screwdriver (STC200CN2; Tohnichi, Tokyo, Japan)
- Periotest machine (Periotest M; Medizintechnik Gulden e.K., Modautal, Germany)
- Light polymerized resin (Technovit 7200 VLC, Heraeus, Kulzer GmbH Division Technique, Wehrheim, Germany)
- Diamond disc microtome (Leica SP1600; Leica microsystems, Nussloch, Germany)
- Bright-field microscopy (Nikon ECLIPSE LV100N POL; Nikon instrument Inc., NY, USA)

- Laser confocal microscopy (TCS SP8; Leica Microsystems CMS GmbH, Mannheim, Germany)
- A vice table
- Micromotor
- A carborundum disc
- Waterproof sandpapers No. 80, 150, 320, 480, 600, 800, 1000, 1500, 2000
- A vernier caliper
- Ethanol
- Vacuum chamber
- Vacuum pump
- Microscope slides
- 24 × 24 mm cover glass

3.3 Research Methods

3.3.1 Research Plan



3.3.2.1 Sample Size Estimation

The G*Power 3.1 software was employed to estimate the required sample size for the present study. The statistical analysis tool selected for this study is the Wilcoxon-Mann-Whitney test. Based on the effect size value of 2.4, which was calculated using means and standard deviations from a relevant prior study (106) that evaluated microcrack surface density using two different dental implant pitch sizes with a similar taper-cylindrical shape, and with a type I error (α) value of 0.05, type II error value of 0.95, and a number of groups value of 3, the total sample size was computed to be 14 (Figure 5)

Accordingly, five orthodontic miniscrews were used in each group of the different designs.

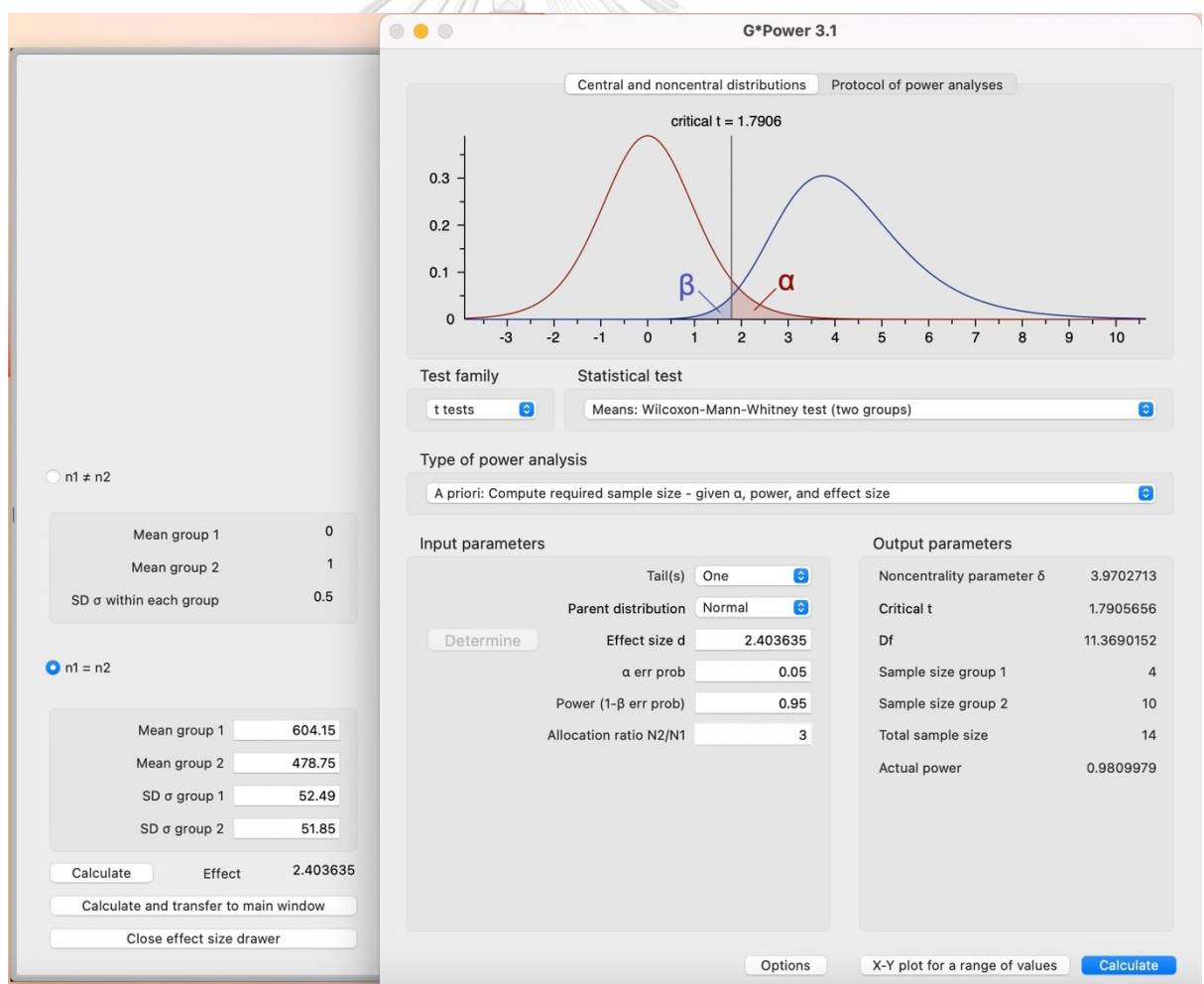


Figure 5 The sample size calculated from the G-power program

3.3.2.2 Preparation of the Bone Specimens

Ethical approval was not required because no animal experiments or human studies were performed during this study. The fresh porcine tibia bones were prepared from an extracted crus, and the mesio-distal ends were removed using a hacksaw. The trabecular bone was removed, and the cortical bone was cut into fifteen 15.0 ×12.0 mm blocks under water irrigation. The blocks were polished using emery paper until they were 1.0-mm thick and had a uniformly flat surface. To confirm the thickness of the specimens, the vernier caliper was used. Two diagonals were drawn from opposite corners of the specimens. The intersection of these lines was determined as the point of miniscrew insertion (Figure 6) The storage process of these bone blocks before orthodontic miniscrew insertion was to deep freeze them in normal saline solution, kept at -20°C. This process has been proven not to undermine bone properties (107).

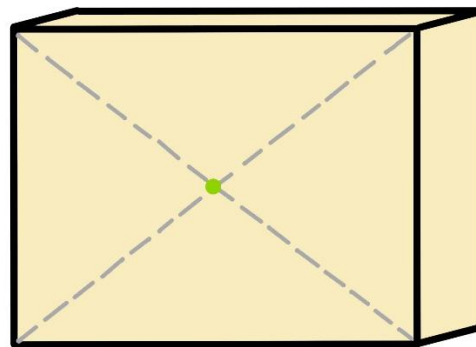


Figure 6 The intersection of two diagonals was the miniscrew insertion point.

3.3.2.3 Miniscrew Insertion test

Orthodontic miniscrews and bone blocks were divided into 3 groups:

Group	Pitch	Height
H _C P _N	0.30 mm.	0.12 mm.
H _C P _C	0.60 mm.	0.12 mm.
H _T P _C	0.60 mm.	0.36 mm.

Thread pitches were varied into 2 sizes composing with 0.3 and 0.6 mm and thread heights were varied into 2 sizes composing with 0.12 and 0.36 mm. Using these designs, all the other thread shapes remained consistent across each group. However, despite having a comparable outer diameter, the H_CP_C group exhibited a larger core diameter compared to the H_TP_C group due to the variation in thread height.

The bone blocks were thawed, then secured in the vice table (Figure 7). A 0.7 mm diameter pilot hole was drilled into the center of each block under water irrigation. The orthodontic miniscrews from each group were inserted until the distance between the neck and the cortical bone was about 1 mm. Maximum insertion torque was recorded in the placement procedure with digital torque screwdriver to evaluate the primary stability (Figure 8). At the time of screw insertion, the final gap between the bone block surface and the bottom of the orthodontic miniscrew head was maintained at 1.0 mm.



Figure 7 The vice table.

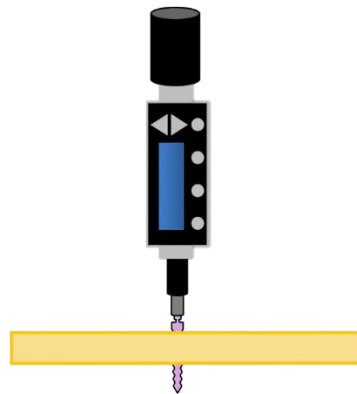


Figure 8 Orthodontic miniscrew was inserted into the porcine tibia bone block while measuring the insertion torque with digital torque screw driver

3.3.2.4 Periotest

To evaluate the stability against a lateral load, the miniscrew degree of mobility was recorded by the Periotest machine. While testing, the rod and test surface was perpendicular to each other. The result of this test was indicated by the Periotest values (PTVs) varied from -8 (lowest mobility) to +50 (highest mobility). The mean Periotest values from 3 times measurement in all four directions at 90° intervals was calculated.

3.3.2.5 Bulk Staining with Basic Fuchsin

All of bone blocks were stored in 70% ethanol for 48 hours before starting the staining process. They were bulk stained with 1% basic fuchsin in a graded series of ethanol under vacuum according to the standard protocol as following (91).

- 1% basic fuchsin in 80% ethanol.
- Change solution.
- 1% basic fuchsin in 80% ethanol.
- Repeat steps 1-3, 1% basic fuchsin in 90% ethanol.
- Repeat steps 1-3, 1% basic fuchsin in 100% ethanol.
- Rinse in 100% ethanol for 1 hour to remove excess stain; the specimen can be shaken or swirled occasionally during rinse.

Thereafter, these blocks were embedded in light polymerized resin and sectioned parallel to the plane of orthodontic miniscrew axis and also the longitudinal axis of the bone specimen to approximately 40-60 μm . (Figure 9)

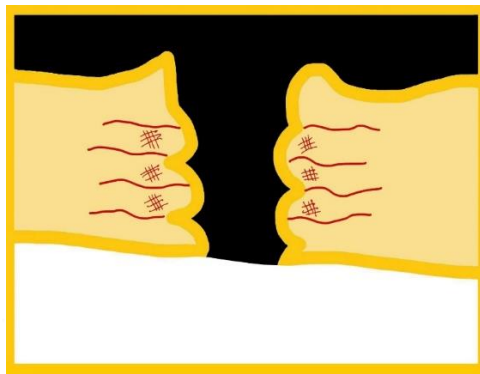


Figure 9 The bone block will be sectioned parallel to the plane of orthodontic miniscrew axis.

3.3.2.6 Measurement of Bone Microdamage

The laser confocal microscopy was performed to visualize the bone microdamage in the following parameters.

a) *Total crack length (TCL)*: the summation of all linear crack length measured between the starting point of crack generated within 0.5 mm from the outer diameter of the orthodontic miniscrew to the ending point of the crack. This parameter represented the extent of the linear damage progression (Figure 10)

This parameter was used in the measurement of linear crack (Figure 11) which was defined as the defect with sharp border staining (103) that extended about 100 μm (99, 108).

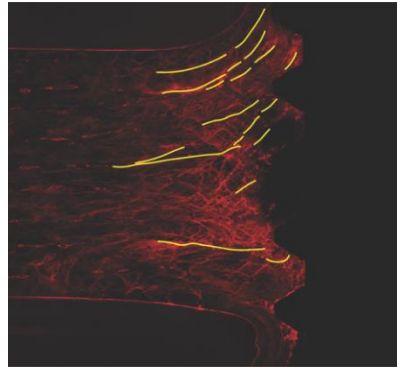


Figure 10 The linear crack measurement method. The yellow lines illustrate the quantitative length of the linear cracks. The total crack length (TCL) is the summation of all linear crack lengths representing the extent of linear damage progression.

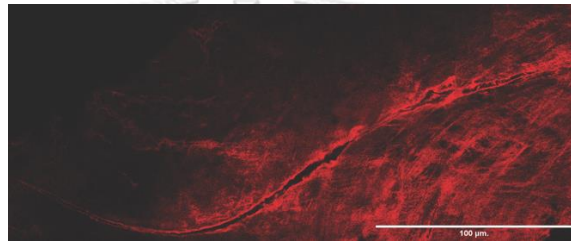


Figure 11 The linear microcrack typically extended about 100 μm .

b) *Total damage area (TDA)*: This value indicates the extent of linear cracks and diffuse damage. Using the histological sections, the area including all linear crack and diffuse damage was defined as the region of interest. The image of the region was converted to an 8-bit format and binarized using a threshold ≥ 36 and particle size ≥ 0 . After this procedure, the damaged area (shown in black) was calculated as the TDA (Figure 12) The diffuse microdamage was identified as a fine network of crack in submicron level (Figure13) (85). Both microdamage types were count together since both types have shown their ability to impair bone mechanical properties (86, 88).

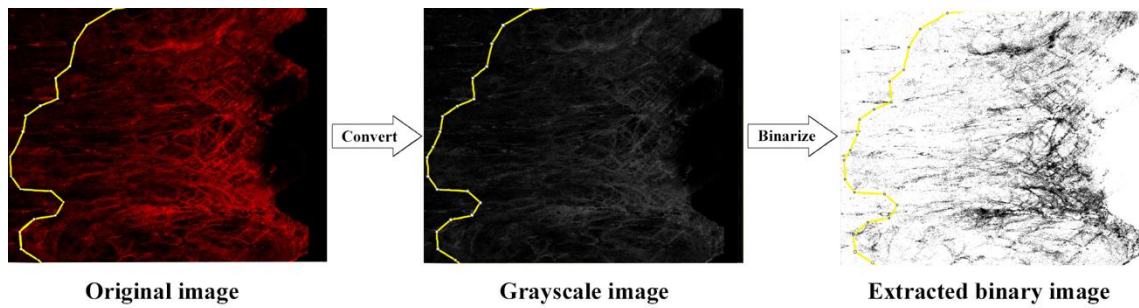


Figure 12 The procedure was performed to extract the damaged areas using the Image J Fiji program. The total damage area (TDA) represents all diffuse microdamage and the border staining of the linear cracks indicating all microdamage extent.

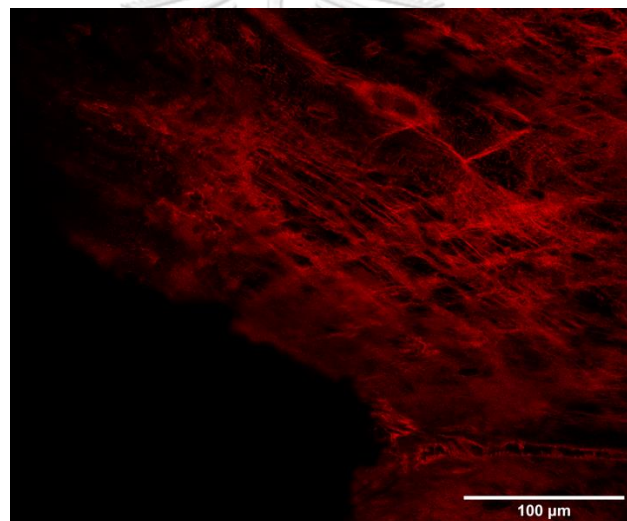


Figure 13 The diffuse microdamage is the region of submicron-size crack that is too small to be distinguished from one another.

3.3.2.7 Quantitative evaluation of the inserted state

To quantitatively evaluate the inserted state of the orthodontic miniscrew in the cortical bone, histological sections were observed using bright-field microscopy and NIS elements imaging software. The acquired images were analyzed using Image J analysis software on these 2 parameters;

Surface length (SL): This value was the length of miniscrew surface engaged in the cortical bone (Figure 14)

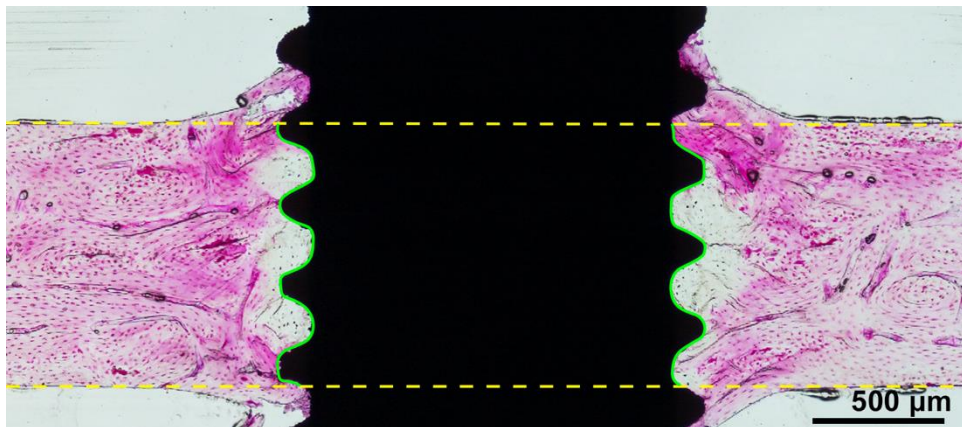


Figure 14 Both sides of miniscrew surface length that would be counted are represented by the green line.

Bone compression area (BCA): The amount of bone areas that were compressed by orthodontic miniscrew while insertion. To calculate the area of bone compression, the miniscrew area in cross-section of the bright-field histological picture was deducted with the area of the pilot hole ($0.7 \times 1.0 \text{ mm}^2$) (Figure 15).

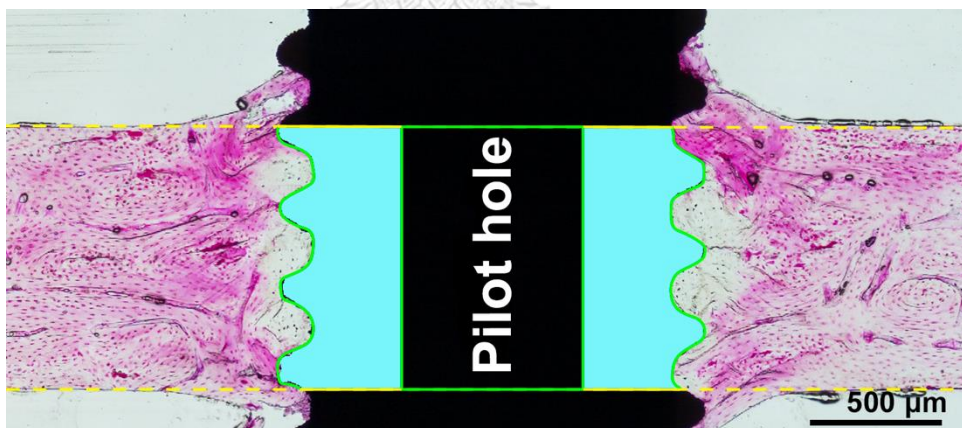


Figure 15 The blue area represents the area that would be compressed by the orthodontic miniscrew while insertion after the predrilling would be done.

The area of compressed bone per surface unit was calculated as the ratio between the BCA and SL.

3.4 Statistical Analysis

3.4.1 Data Analysis According to Different Miniscrew Geometric Design

- Maximum insertion torque (N.cm)
- Periotest value
- Total crack length (mm)
- Total damage area (mm²)
- Surface length (mm)
- Bone compression area (mm²)
- The area of compressed bone per surface unit

3.4.2 Statistical Analysis

The bone microdamage parameters were calculated using image analysis software (Image J; WS Rasband, National Institutes of Health). The data from the insertion test, stability test, geometric factors of the orthodontic miniscrew, and bone microdamage were analyzed using multiple comparisons of Bonferroni-corrected Wilcoxon's rank-sum test. Statistical significance was set at $P < 0.05$. . To assess the reliability of bone microdamage measurements, a reliability test was performed by a single examiner who measured the samples twice, with a one-month interval between measurements. The intra-rater reliability was evaluated using the intraclass correlation coefficient. The statistical analyses were performed using "R" software (version 4.0.2; <http://www.r-project.org/>; accessed on June 25, 2020).

CHAPTER IV RESULTS

4.1 Insertion test

The MIT of the $H_C P_N$ orthodontic miniscrews was significantly greater than that of the other miniscrews ($P < 0.05$). In contrast, the MIT of the $H_C P_C$ and $H_T P_C$ orthodontic miniscrews were similar (Figure 16A).

4.2 Stability test

Using the Periotest system, the PTVs of the $H_C P_N$ and $H_T P_C$ orthodontic miniscrews were significantly greater than those of the $H_C P_C$ ($P < 0.05$) orthodontic miniscrews. However, although the $H_T P_C$ orthodontic miniscrews had a greater PTV than the $H_C P_N$ orthodontic miniscrews, the difference was not significant ($P > 0.05$) (Figure 16B).

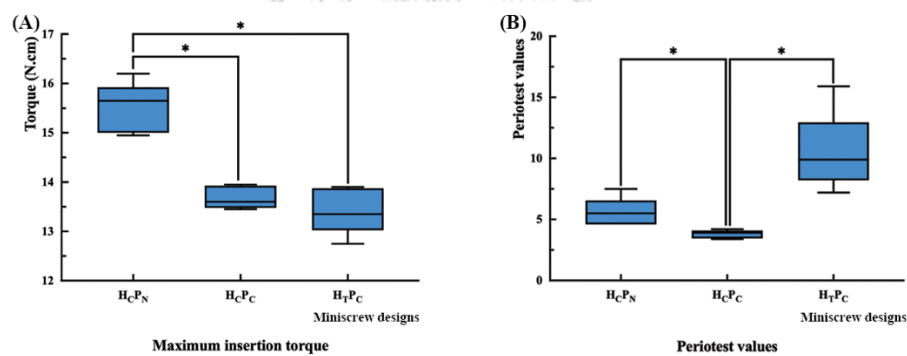


Figure 16 Box-and-whisker graphs demonstrating the mean and median values. (A) The maximum insertion torque of the different orthodontic miniscrew geometric designs. (B) The Periotest value of the different orthodontic miniscrew geometric designs. *Significant differences between each pair of plots ($P < 0.05$).

4.3 Bone microdamage

Linear cracks and diffuse damage were observed histologically, particularly at the tip of the orthodontic miniscrew threads. The linear cracks did not extend 1.0 mm beyond the surface of the miniscrew. Diffuse damage was found adjacent to the orthodontic miniscrew surface and around the linear cracks (Figure 17).

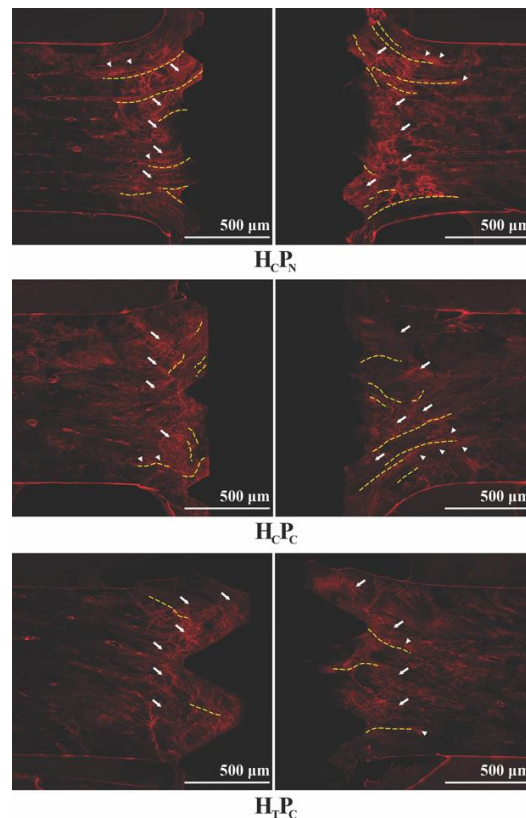


Figure 17 Bone microdamage images obtained using laser confocal microscopy. The upper panel shows bone microdamage around the H_cP_n orthodontic miniscrew on the left and right sides. The middle panel shows bone microdamage around the H_cP_c orthodontic miniscrew on the left and right sides. The lower panel shows bone microdamage around the H_tP_c orthodontic miniscrew on the left and right sides. The yellow dashed lines indicate the linear cracks. The diffuse microdamage adjacent to the orthodontic miniscrew surface appeared as a network of fine cracks that cannot be discriminated (white arrow). The diffuse microdamage was also found attached to the surface of the linear crack (white arrowhead), which presented as a black space surrounded by the basic fuchsin-stained area.

The bone microdamage measurements demonstrated excellent reliability, as indicated by the intraclass correlation coefficient, which ranged from 0.933 to 0.998.

The H_cP_n orthodontic miniscrews had the highest TCL and TDA values, followed by those of the H_cP_c and H_tP_c orthodontic miniscrews. Furthermore, a

significant difference in TDA was observed between the $H_C P_N$ and $H_T P_C$ orthodontic miniscrews ($P < 0.05$); however, no significant differences were observed between the TCL or TDA of the other groups ($P > 0.05$) (Figure 18A, Figure 18B).

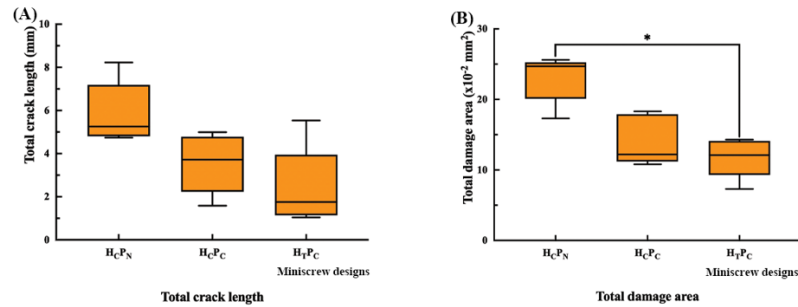


Figure 18 Box-and-whisker graphs demonstrating the mean and median values. (A) The total crack length of the different orthodontic miniscrew geometric designs. (B) The total damage area of the different orthodontic miniscrew geometric designs.

*Significant differences between each pair of plots ($P < 0.05$).

4.4 Quantitative evaluation of the inserted state

The histological evaluation revealed that the orthodontic miniscrew and cortical bone were in contact; a contact rate of almost 100% was observed for all specimens. The $H_C P_N$ orthodontic miniscrews had the highest BCA value followed by the $H_C P_C$ and $H_T P_C$ orthodontic miniscrews. Significant differences were observed between each group ($P < 0.05$). The $H_T P_C$ orthodontic miniscrews had the highest SL value, followed by the $H_C P_N$, and $H_C P_C$ orthodontic miniscrews. Significant differences were observed between each group ($P < 0.05$) (Figure 19A, Figure 19B). The area of compressed bone per surface unit of the $H_C P_N$ and $H_C P_C$ groups orthodontic miniscrews were significantly larger than that of the $H_T P_C$ ($P < 0.05$) orthodontic miniscrews. However, the area of compressed bone per surface unit of the $H_C P_N$ and $H_C P_C$ orthodontic miniscrews were similar (Figure 20).

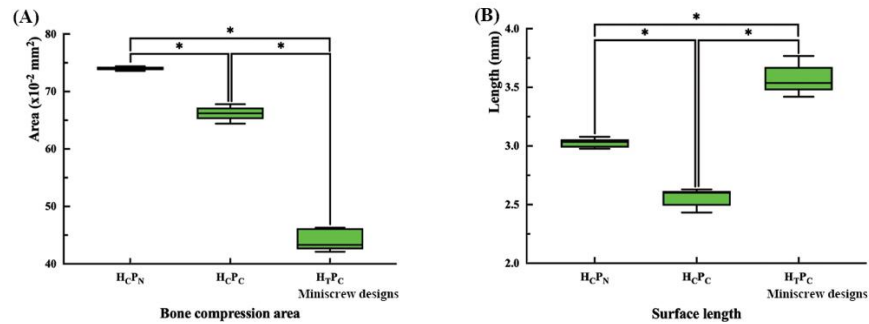


Figure 19 Box-and-whisker graphs demonstrating the mean and median values. (A) The bone compression area of the different orthodontic miniscrew geometric designs. (B) The surface length of the different orthodontic miniscrew geometric designs. *Significant differences between each pair of plots ($P < 0.05$).

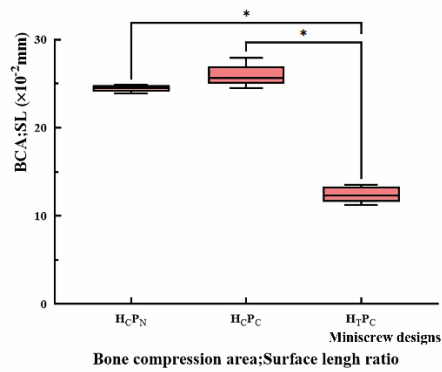


Figure 20 Box-and-whisker graphs demonstrating the mean and median values of the area of compressed bone per surface unit. *Significant differences between each pair of plots ($P < 0.05$).

CHAPTER V DISCUSSION

This study examined the effect of orthodontic miniscrew geometry on their primary stability after insertion. To evaluate the primary stability, the insertion torque and PTV were analyzed. The insertion torque is the most common index used to evaluate the stability of orthodontic miniscrews and is based on the evaluation of dental implants, which have a geometry similar to that of orthodontic miniscrews (109). However, the head region of orthodontic miniscrews is exposed on the mucosa after insertion, which sets them apart from dental implants (68). Due to the lateral loading exerted on the head region of orthodontic miniscrews by the surrounding soft tissues and food bolus (8), the present study also evaluated their PTV.

The accuracy of the PTV is debatable according to a previous study (110). A loading test using a universal testing machine that generated a stress–strain curve was considered due to its high reliability. However, to generate the stress–strain curve, the test must be performed until the specimen is plastically deformed (111). Therefore, the bone specimen microdamage cannot be evaluated after the loading test. In the present study, variations in the mechanical properties of the bone pieces could not be eliminated because the specimens were from experimental animals. To minimize variability in the results, the PTV and bone microdamage were evaluated using the same specimens. The loading during the Periotest procedure is small and intermittent enough to be used for clinical follow-up (112). Furthermore, the Periotest can evaluate bone microdamage and stability using the same specimens, making it suitable for the stability test in this study.

The stability against lateral load is affected by the stiffness of the orthodontic miniscrew, the mechanical properties of the test sample, and the contact area between the orthodontic miniscrew and the test sample (113, 114). In our study, the stiffness of the orthodontic miniscrew was different in each group due to the different orthodontic miniscrew geometries. However, the Ti6Al4V alloy orthodontic miniscrew is stiffer and less deformable than the animal-derived cortical bone test sample. Therefore, the disparity in stiffness between each orthodontic miniscrew group does not need to be considered when evaluating the PTV.

We used cortical bone tissue as the test sample because primary stability is acquired mainly from this tissue (58). Synthetic cortical bone tissue and bone from experimental animals were also considered as candidates for the test samples. Synthetic cortical bone has uniform mechanical properties; however, its mechanism of microdamage generation is different from that of real bone (115). Moreover, whether the microdamage-induced changes in mechanical properties could be simulated was not clear. Because the mechanical properties of microdamage to cortical bone tissue had to be simulated to evaluate the PTV, cortical bone obtained from experimental animals was used.

Among several animal species, the minipig was selected because its bone density, degree of calcification, healing process, and remodeling activity are similar to those of humans (116). To obtain as many bone pieces as possible from a single extracted bone, we selected a tibia that was larger than the jawbone. Furthermore, its flat surface served to accentuate the impact of the thread pitch and thread height on the bone.

The use of the tibia to acquire bone pieces for our study presented two issues. The first issue pertained to the pilot hole. The orthodontic miniscrews in our study had a cutting flute and could be inserted using a self-tapping and self-drilling procedure. However, some of the orthodontic miniscrew tips broke during insertion without a pilot hole during our preliminary experiment. Therefore, we had to prepare a pilot hole with a minimum diameter that was the same as that of the orthodontic miniscrew tip (0.7 mm). Given that the pilot holes were performed using irrigation, and in light of previous research demonstrating that self-tapping techniques result in less bone microdamage compared with self-drilling techniques (42), it is likely that any bone microdamage resulting from the pilot hole drilling process was minimal. The second issue involved the insertion torque. The overall insertion torque was greater than the optimal insertion torque for humans (5–10 N-cm) (63). These two problems were attributed to the bone mineral density of the tibia being greater than that of the jawbone (117). The greater bone mineral density of the tibia required the use of a pilot hole.

Several studies have used basic fuchsin staining, which is the most common method used to visualize bone microdamage (42, 90, 91, 102). Our preliminary investigation also demonstrated that this method is the most efficient and straightforward approach. This staining technique enables clear visualization of the microdamage line, and the use of laser confocal microscopy minimizes light flaring, allowing the bone separation space within the linear crack to be observed. Furthermore, this method facilitates visualizing the diffuse damage structure, which resembles a network of microcracks, with great clarity.

To quantify differences in orthodontic miniscrew geometry, the BCA and SL were calculated from measurements made using the histological sections. The BCA assay results indicated that the $H_C P_N$ orthodontic miniscrews had a greater value than the $H_C P_C$ orthodontic miniscrews because the $H_C P_N$ design has a greater number of threads. In comparison, the $H_T P_C$ geometry had a lower value than the $H_C P_C$ geometry because the $H_T P_C$ design has a shaft with a smaller diameter because of its tall threads. The $H_C P_C$ orthodontic miniscrews demonstrated the smallest SL value, followed by the $H_C P_N$ and $H_T P_C$ orthodontic miniscrews. This is because the contact area increased as the number or height of the threads increased; the height of the threads was found to be more important than the number of threads (6).

Previous studies that examined the effects of the orthodontic miniscrew design reported that changes in geometry affected the insertion torque (7, 44, 49). Regarding the geometric factors in our study, a larger BCA increased the insertion torque. The SL indicates the length of the orthodontic miniscrew surface engaged in the cortical bone tissue and can be regarded as synonymous with the surface area of the orthodontic miniscrew in that region. Therefore, a greater SL increases friction during insertion, which also increases the insertion torque. The $H_C P_N$ geometry had a greater BCA and SL than the $H_C P_C$ geometry and contributed to a greater MIT in the $H_C P_N$ design. The $H_T P_C$ geometry had a smaller BCA, but greater SL, compared with the $H_C P_C$ geometry; these differences contributed to the similarity of the MIT of the $H_T P_C$ and the MIT of the $H_C P_C$ designs.

To our knowledge, there are no reports on the effect of orthodontic miniscrew geometry on bone microdamage. However, it is expected that a greater

area of compressed bone per surface unit results in a higher occurrence of bone microdamage. The area of compressed bone per surface unit was estimated by dividing the BCA by the SL. Compared with the $H_C P_C$ orthodontic miniscrews, the $H_C P_N$ orthodontic miniscrews had similar values. Furthermore, the $H_T P_C$ orthodontic miniscrews had values that were approximately half those of the $H_C P_C$ orthodontic miniscrews. Based on these results, the $H_C P_N$ and $H_C P_C$ orthodontic miniscrews should cause the same level of bone microdamage, and the $H_T P_C$ design should cause less bone microdamage. However, the TCL and TDA measurement results indicated that the $H_C P_N$ design had the largest values, followed by those of the $H_C P_C$ and $H_T P_C$ orthodontic miniscrews. These findings indicate that the bone microdamage caused by the $H_C P_N$ orthodontic miniscrews was greater than expected. These results can be explained by the histological observation of the bone microdamage primarily at the tip of the threads. Although the BCA-to-SL ratios of the $H_C P_N$ and $H_C P_C$ orthodontic miniscrews were similar, the amount of bone microdamage increased with the $H_C P_N$ orthodontic miniscrews because of the greater number of threads.

Regarding the contact area between the orthodontic miniscrew and cortical bone tissue, the histological evaluation revealed that bone contact rate of all specimens was almost 100%. Therefore, the SL directly represented the contact area between the orthodontic miniscrew and test sample. A negative relationship between the contact area and mobility of the orthodontic miniscrew was expected; however, the direct relationship was observed based on the SL and PTV results. In contrast, there was a negative relationship between BCA and PTV comparing $H_C P_N$ and $H_C P_C$ versus $H_T P_C$. This is because the cortical bone tissue was condensed by the lateral load during insertion. Bone condensation is a method used to improve bone quality before dental implant placement (118). Using this technique, the bone is compressed laterally by gradually increasing the indenter size, which increases bone density and improves the primary stability of dental implants (119). However, to confirm this hypothesis, further investigation into the bone density surrounding the orthodontic miniscrew is necessary. Despite having a larger BCA, the $H_C P_N$ orthodontic miniscrews had a greater PTV than the $H_C P_C$ orthodontic miniscrews. Furthermore, the $H_C P_N$ orthodontic miniscrews had greater TCL and TDA values than the $H_C P_C$

orthodontic miniscrews with regard to bone microdamage. A possible explanation for these findings is that excessive bone compression increases bone microdamage, which would decrease stability.

With a consistent outer diameter of 1.6 mm in this study, the inner diameter was comparable between the $H_C P_N$ and $H_C P_C$ groups due to their similar thread height. However, the $H_C P_C$ group had a larger inner diameter than the $H_T P_C$ group, likely due to its greater thread height (Table 1). This discrepancy can be attributed to varying degrees of bone compression. Further research is needed to explore the impact of thread height by examining different thread heights while maintaining a consistent inner diameter.

	Outer diameter (mm)	Inner diameter (mm)
$H_C P_N$	1.6	1.36
$H_C P_C$	1.6	1.36
$H_T P_C$	1.6	0.88

Table 1 The orthodontic miniscrews outer diameters and inner diameters

The MIT and PTV results indicated that the $H_C P_N$ and $H_C P_C$ designs had superior primary stability compared with the $H_T P_C$ design. Although the primary stabilities of the $H_C P_N$ and $H_C P_C$ designs were similar, the $H_C P_C$ design was associated with less bone microdamage. Because bone microdamage adversely affects cortical bone metabolism, the $H_C P_C$ design has the greatest potential for clinical use. Moreover, if the geometry of the thread tip can be modified to avoid the concentration of bone microdamage, then the primary stability of the orthodontic miniscrew can be further improved.

Our findings have certain limitations when it comes to clinical applicability. The experimental procedure was conducted on animal bones that have a density that is dissimilar to that of human bones. Furthermore, the manufacturer has only disclosed the outer diameter sizes and orthodontic miniscrew length, with no information being provided on the thread height. This is a crucial aspect to consider because a larger thread height correlates with a smaller inner diameter, and if the

inner diameter is smaller than the pilot hole size, there could potentially be a gap between the inner surface and the bone. . Moreover, this investigation was limited to the examination of the smallest and largest commercially accessible thread height sizes (7, 120). To validate the clinical applicability of orthodontic miniscrews with a diameter of 1.6 mm, it is necessary to carry out further research encompassing a wider range of thread height sizes. It is therefore imperative to exercise caution in this regard.



CHAPTER VI CONCLUSION

A narrower pitch of the orthodontic miniscrew increases the number of threads per length unit and the compressed area of the cortical bone tissue, resulting in increased bone microdamage during insertion. Increased bone microdamage leads to decreased primary stability.

An increased thread height with the same outer diameter decreased the compressed area of the cortical bone tissue, resulting in less bone microdamage. However, even if bone microdamage is reduced, inadequate cortical bone tissue compression leads to decreased primary stability.

Nonetheless, it is imperative to consider the clinical significance of the impact exerted by the thread pitch and thread height on the success rate of orthodontic miniscrew insertion. To substantiate the findings, further investigation into the clinical success rate of orthodontic miniscrews will be warranted.

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