Histological Evaluation and Inflammatory Response of Different Abutment Materials: An Experimental Study in Human

Miss Teeratida Sampatanukul

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Esthetic Restorative and Implant Dentistry
Faculty of Dentistry
Chulalongkorn University
Academic Year 2015

การศึกษาลักษณะทางจุลกายวิภาค และปฏิกิริยาการอักเสบของเนื้อเยื่อรอบหลักยึดที่ทำจากวัสดุต่างชนิดในมนุษย์

นางสาวธีรธิดา สัมปทานุกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาทันตกรรมบูรณะเพื่อความสวยงามและทันตกรรมรากเทียม
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

หัวข้อวิทยานิพนธ์	การศึกษาลักษณะทางจุลกายวิภาค และปฏิกิริยาการอักเสบของเนื้อเยื่อรอบหลักยึดที่ทำจากวัสดุต่างชนิดในมนุษย์
โดย	นางสาวธีร์ธิดา สัมปทานุกุล
สาขาวิชา	ทันตกรรมบูรณะเพื่อความสวยงามและทันตกรรมรากเทียม
อาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก	ผู้ช่วยศาสตราจารย์ ทันตแพทย์ ดร. อาทิพันธุ์ พิมพ์ขาวขำ
อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม	รองศาสตราจารย์ แพทย์หญิง วรนุช ธนากิจ

คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย อนุมัติให้นับวิทยานิพนธ์ฉบับนี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญามหาบัณฑิต

--คณบดีคณะทันตแพทยศาสตร์

(ผู้ช่วยศาสตราจารย์ ทันตแพทย์ ดร. สุชิต พูลทอง)

คณะกรรมการสอบวิทยานิพนธ์

--ประธานกรรมการ

(รองศาสตราจารย์ ทันตแพทย์ เฉลิมพล ลี้ไวโรจน์)

--อาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก

(ผู้ช่วยศาสตราจารย์ ทันตแพทย์ ดร. อาทิพันธุ์ พิมพ์ขาวขำ)

--อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม

(รองศาสตราจารย์ แพทย์หญิง วรนุช ธนากิจ)

--กรรมการภายนอกมหาวิทยาลัย

(ศาสตราจารย์ นายแพทย์ วรชัย ศิริกุลชยานนท์)

ธีรธิดา สัมปทานุกุล : การศึกษาลักษณะทางจุลกายวิภาค และปฏิกิริยาการอักเสบของเนื้อเยื่อรอบหลักยึดที่ทำจากวัสดุต่างชนิดในมนุษย์ (Histological Evaluation and Inflammatory Response of Different Abutment Materials: An Experimental Study in Human) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ทพ. ดร. อาทิพันธุ์ พิมพ์ขาวขำ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. พญ. วรนุช ธนากิจ, 80 หน้า.

วัตถุประสงค์: เพื่ออธิบาย และเปรียบเทียบผลของการประเมินการยึดเกาะของเนื้อเยื่อรอบหลักยึดและปฏิกิริยาการอักเสบของเนื้อเยื่อรอบหลักยึดต่อหลักยึดที่ทำจากวัสดุต่างกัน 3ชนิด ภายหลังการฝังรากเทียม 8 สัปดาห์

วิธีการศึกษาวิจัย: รากเทียมทั้งสิ้น 15ซี่ ได้รับการฝังและใส่หลักยึด 3ชนิด ได้แก่ ไทเทเนียม เซอร์โคเนีย และโลหะผสมทอง แบบสุ่ม แต่กลุ่มมีจำนวน 5 ซี่ เนื้อเยื่อรอบหลักยึดถูกตัดเพื่อศึกษาทางจุลชีววิทยาที่ 8 สัปดาห์หลังการฝังรากเทียม เนื้อเยื่อดังกล่าวถูกนำไปฝังในเรซิน ผ่านกระบวนการเตรียมสไลด์ และย้อมด้วยสีเฮช แอนด์ อี (H & E) เนื้อเยื่อรอบหลักยึดได้รับการประเมินในขั้นตอนทางคลินิก โดยใช้ดัชนีเหงือก (Gingival index) ขั้นตอนการผ่าตัด โดยใช้คะแนนผ่าตัด (Surgical score) และขั้นตอนการตรวจชิ้นเนื้อ โดยใช้ร้อยละการยึดเกาะของเนื้อเยื่อ (Attachment percentage) และประเมินการตอบสนองต่อการอักเสบ โดยใช้เกรดขอบเขตการอักเสบ (Inflammatory extent grade) และ เกรดจำนวนเซลล์อักเสบ (Inflammatory cellularity grade)

ผลการวิจัย: เนื้อเยื่อรอบหลักยึดรากเทียมของกลุ่มโลหะผสมทองทุกซี่ได้รับคะแนนดัชนีเหงือก เท่ากับ 1 ซึ่งแตกต่างจากกลุ่มอื่น แต่การทดสอบทางสถิติไม่แสดงให้เห็นความสัมพันธ์ระหว่างคะแนนดัชนีเหงือกกับชนิดหลักยึด ($p = 0.071$) สำหรับคะแนนศัลยกรรม กลุ่มเซอร์โคเนียมีผลที่ดีกว่าโดยมีคะแนนระดับ 3 จำนวน 0% ในขณะที่ 40% ในกลุ่มโลหะผสมทองได้รับคะแนนระดับ 3 ทั้งนี้ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p = 0.262$) สำหรับร้อยละการยึดเกาะของเนื้อเยื่อไททาเนียมและเซอร์โคเนีย แสดงค่าเฉลี่ยร้อยละการยึดเกาะของเนื้อเยื่อใกล้เคียงกัน ในขณะที่โลหะผสมทองมีค่า ร้อยละการยึดเกาะของเนื้อเยื่อต่ำกว่ามาก ซึ่งพบว่ามีผลอย่างมีนัยสำคัญทางสถิติ ($p = 0.004$) สำหรับเกรดขอบเขตการอักเสบและเกรดจำนวนเซลล์อักเสบ โอกาสที่จะได้คะแนนสูงขึ้นหนึ่งระดับของหลักยึดโลหะผสมทองเป็น 5.18 และ 17.8 เท่าของหลักยึดไทเทเนียมตามลำดับ และโอกาสที่จะได้คะแนนสูงขึ้นหนึ่งระดับของหลักยึดเซอร์โคเนียเป็น 0.87 เท่า และ 7.5 เท่าของหลักยึดไทเทเนียมตามลำดับ

สรุปผลการวิจัย: ที่ระยะเวลาหลังฝังรากเทียม 2 เดือน เนื้อเยื่อรอบหลักยึดรากเทียมชนิดโลหะผสมทองมีการยึดของเนื้อเยื่อเหงือกต่อหลักยึดด้อยกว่าหลักยึดรากเทียมชนิดไททาเนียมและเซอร์โคเนีย การอักเสบมีแนวโน้มที่จะพบในเนื้อเยื่อรอบหลักยึดรากเทียมชนิดโลหะผสมทองมากกว่าหลักยึดรากเทียมชนิดไททาเนียมและเซอร์โคเนีย

สาขาวิชา	ทันตกรรมบูรณะเพื่อความสวยงามและทันตกรรมรากเทียม	ลายมือชื่อนิสิต	....
		ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
ปีการศึกษา	2558	ลายมือชื่อ อ.ที่ปรึกษาร่วม	....

5675830932 : MAJOR ESTHETIC RESTORATIVE AND IMPLANT DENTISTRY
KEYWORDS: ABUTMENT, GOLD ALLOY, HISTOLOGY, IMPLANT, PERI-IMPLANT SOFT TISSUE, RESIN EMBEDDED, TITANIUM, ZIRCONIA

TEERATIDA SAMPATANUKUL: Histological Evaluation and Inflammatory Response of Different Abutment Materials: An Experimental Study in Human. ADVISOR: ASST. PROF. ATIPHAN PIMKHAOKHAM, Ph.D., CO-ADVISOR: ASSOC. PROF. VORANUCH TANAKIT, 80 pp.

Objective: To describe and compare effects of 3 different abutment materials on the attachment evaluation and inflammatory reactions of the soft tissue around abutments at 8 weeks healing period.

Material and Methods: Fifteen posterior edentulous areas were treated with implant restorations. Three types of abutment materials; titanium, zirconia, and gold alloy, were randomly inserted on implant fixtures on the surgery day, 5 abutments of each group. Tissue biopsies from peri-implant tissue around the abutments were harvested at 8 weeks after implant surgery. The specimens were processed using non-separation resin embedded technique and stained with H&E. The characteristics of peri-implant tissue attachment were assessed at clinical stage using gingival index (GI) score, surgical stage (surgical score) and histological stage (attachment percentage). And the inflammatory responds were evaluated using inflammatory extent grade and inflammatory cellularity grade.

Results: All cases of gold alloy group received GI score equal 1, but chi-square test suggests no association between GI score and abutment type ($p = 0.071$). For Surgical score, zirconia had a better result with 0% of score 3, while 40% in gold alloy group received score 3, but no statistically significant differences were found among groups ($p = 0.262$). For attachment percentage, titanium and zirconia abutments exhibited almost similar mean attachment percentages while gold alloy abutments received much lower mean percentage. A significant effect on attachment percentage was found among 3 groups ($p = 0.004$). For inflammatory extent grade and inflammatory cellularity grade, the odds of being one grade higher for gold alloy abutment was 5.18 and 17.8 times that of titanium abutment, respectively. For inflammatory extent grade of zirconia abutment, the odds was 0.87 times lower, and for inflammatory cellularity the odds was 7.5 times higher than that of titanium group.

Conclusions: At 2 months haling period, peri-implant tissue around gold alloy abutments resulted in poorer attachment condition compare to titanium and zirconia abutments. Inflammation tended to be higher in tissue around gold alloy abutments than titanium and zirconia abutments.

Field of Study:	Esthetic Restorative and Implant Dentistry	Student's Signature	....
		Advisor's Signature	....
Academic Year:	2015	Co-Advisor's Signature	....

กิตติกรรมประกาศ

First and foremost, I wish to express my deep gratitude to my advisors, Associate Professor Atiphan Pimkhaokham, Assistant Professor Pravej Serichetaphongse and Associate Professor Voranuch Thanakit. Their kindly supports, inspiring suggestions, enthusiastic encouragement and useful critiques of this research, have been precious for the development of this thesis.

I would like to express my great appreciation to my thesis committee, Associate Professor Chalermpol Leeviroj and Professor Vorachai Sirikulchayanonta, for their valuable and constructive suggestions. Their willingness to give their time so generously has been very much appreciated.

Moreover, I would also like to thank Professor Prasit Pavasant and the staffs of the oral biology laboratory for enabling me to visit their offices to observe their operations, and taught me all the laboratory works. Without their helps this work could not have been completed.

Also, I would like to thank Associate Professor Cameron Pual Hurst, for his advice on statistical parts and help me through all statistical analysis. My grateful thanks are also extended to Assistant Professor Tewarit Somkotra and Mr. Siwachoat Srisuttiyakorn for their help in statistical explanation and data management.

Most of all, I would like to acknowledge staffs and friends at Esthetic Restorative and Implant Clinic, especially, Miss Thayika Saruttichart, who helped me with thesis works, and Mr. Kornpong Suthattakul, who supported me and helped correcting my english. And deepest thanks to Mom, Dad, and my brother for their constant love and supports.

Finally, I appreciate the financial support from the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund).

สารบัญ

หน้า

Figure Legends

Table Legends

CHAPTER 1
INTRODUCTION

Rationale and Significance of the Problem

As implants are gaining popularity, at the moment, as a preferable treatment option to substitute missing teeth, studies on implant material's properties and designs has gained momentum among researchers to improve and develop the implant materials chemistry, surface topography and connection designs that would achieve better biological tissue integration with esthetically appealing.

Unlike a tooth which has perpendicular fibers inserted to a cementum layer, the orientation of collagen fibers around implants are mostly circular and parallel to the implant surface, which consider weaker and can be easily invaded by prosthetic cement or oral bacteria [1, 2]. Thus, improvement of attachment of soft tissue to the abutment part results in more stable peri-implant conditions [3].

In an attempt to improve esthetic appearances of gingival area around teeth restoring with titanium implants, abutment materials, with different color, have been used to enhance light reflection through the gingival area [4, 5]. Zirconia and gold alloy abutments resulted in better esthetic appearances, comparing with titanium abutments, especially in the case that a patient had thin gingival biotype and a high smile line.

In addition to the preferring appearance, zirconia abutments were report to have many beneficial effect, such as, less plaque accumulation [6] and shallower probing depth compared to titanium abutment [7]. While, controversial issues were reported in studies using gold alloy abutments. In animal models, on one side, gold alloy abutment had an effect on the dimension of soft tissue and longer observations found reduction of the level of soft tissue and crestal bone [8]. However, on the other side, similar soft tissue dimension was observed when soft tissue healed toward gold alloy and titanium abutments [9].

To date, data on peri-implant tissues derived from human subjects are still not widely obtained. In animal models, biopsy specimens usually contained a block of an implant in bone and soft tissue, which allowed direct observations of histomorphology of structures around an implant [8-11]. Tissue harvesting protocols in human were limited to mostly soft tissue. Moreover, surgical attempt in harvesting peri-implant tissue could affect the tissue morphology and interface including tissue separation and deposition [12, 13]. Therefore, this study aimed to demonstrate routine tissue obtaining by surgical blade and tissue processing by resin embedding technique, in order to assess and evaluate the histology soft tissue attachment to three different implant abutments.

Research Question

Do soft tissue around 3 types of abutments: titanium, zirconia, and gold alloy, exhibit similar responses in term of attachment evaluation and inflammatory reaction?

Objectives of the Study

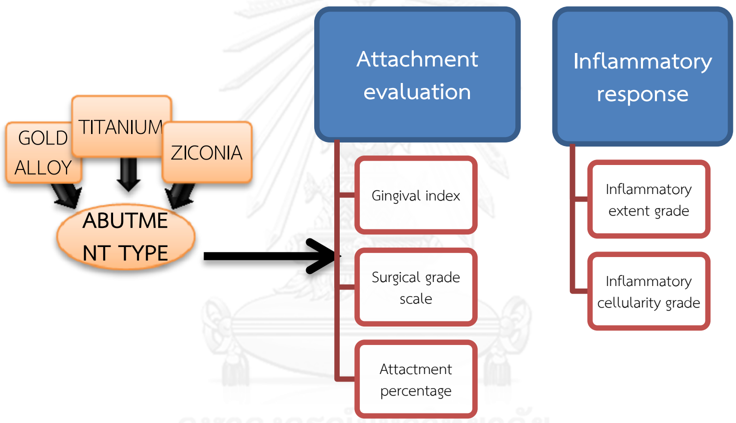
To describe and compare the effects of 3 different types of abutments: titanium, zirconia, and gold alloy, on the attachment evaluation and inflammatory reaction of the soft tissue around the abutments.

Statement of Hypothesis

Soft tissue around 3 types of abutments: titanium, zirconia, and gold alloy, exhibit similar responses in term of attachment evaluation and inflammatory reaction.

Conceptual Framework

Figure 1 Diagram of the conceptual framework



Basis Assumptions

Every implant operators were assumed to perform the same standard technique with equal clinical skills and knowledge.

Study Limitations

The study was the preliminary study to evaluate the peri-implant attachment and inflammatory respond around the 3 types of abutment materials; therefore, descriptive statistic was used to analyze the data. Future study could be conduct to confirm the significant results among the whole population.

Keywords

Abutment, Gold alloy, Histology, Implant, Peri-implant Soft tissue, Resin Embedded, Titanium, Zirconia,

The Expected Benefits

The results of this study will be useful for dentists to choose abutments other than standard titanium one, especially when restoring in specific anterior condition. In fact, if the result shows similar or better soft tissue response and similar or less inflammatory reactions in the gold alloy and zirconia experimental groups, gold alloy and zirconia will be restorative choices other than titanium. In addition, the result will illustrate the histology of human soft tissue forming around 3 different experiential abutments, which could be the baseline evidence for future studies of soft tissue dimensions and could emphasize on the knowledge of soft tissue response around the current implant design.

CHAPTER 2

REVIEW OF LITERATURES

Abutment Materials

Abutment part is important in maintaining soft tissue around dental implants. This part should promote soft tissue contact or at least maintain the level of soft tissue, preventing soft tissue recession [3]. Abrahamson, et al, 1988 suggested that the abutment material has an important role in preventing soft tissue recession and bone destruction [10].

Zirconia abutment is currently used to enhance different light reflection through the thin gingival biotype. With the improvement of CAD/CAM technology, there are many commercial products of zirconia abutments in both prefabricated designs and custom made designs. Retrospective study up to 5 years by Ekfeldt, et al, 2011, revealed promising biological results for zirconia abutment [5]. Moreover, some studies found that zirconia abutments promoted less plaque accumulation than that of titanium abutments [3, 6].

One study by van Brakel, et al, 2010, reported no difference in soft tissue health and early bacterial colonization between zirconia and titanium abutments, but the probing depth in zirconia abutments exhibited shallower [7]. Beside the clinical performances of zirconia abutments, information from the histological studies of this material in human is still currently limited.

Gold alloy is another material which is use to cast a UCLA abutment [8-10, 14]. The yellow color of gold enhances the color of the soft tissue, which results in better esthetic outcomes as compared to titanium. Abrahamson and Cardaropoli, 2007, conducted a study in four beagle dogs to compare the healing around experimental implants made of commercially pure titanium and gold alloy [9]. The results reported that osteointegration was achieved in surfaces made of both titanium and gold alloy. Bone to implant contact percentage (BIC %) values were higher in titanium than in gold alloy surfaces. However, the peri-implant soft tissue dimensions were not different in both materials [9].

Clinical study using gold alloy abutments confirmed comparable results of soft tissue conditions with regard to titanium abutments. Vigolo, et al, 2006, conducted 4 years followed up of implants restored with titanium and gold alloy abutments in 20 subjects. Statistical analysis revealed no significant different behaviors of peri-implant marginal bone and of peri-implant soft tissue level of both abutment types [14].

A review literature by Linkeviclus, 2008, investigated whether material types affected peri-implant soft tissue. The study concluded that using gold abutments should not be considered a risk factor for crestal bone loss and soft tissue recession [15].

Implant Biologic Width

A study by Berglundh et al, 1991, which examined histological features of the peri-implant mucosa in dogs and compared with those of the gingiva around teeth, reported that peri-implant mucosa facing abutments consisted of well keratinized oral epithelium, thin barrier epithelium and connective tissue attachment. The mean biologic width was 3.80 mm around implants and 3.17 mm around teeth. The study suggested this biological structure prevented sub-gingival plaque formations and subsequent infections.

Implant biologic width was later emphasized by Abrahamsson in 1996, whose suggested that a certain width of the peri-implant mucosa was required to enable a proper epithelial connective tissue attachment, and if this soft tissue dimension was less than optimal, bone resorption will occur to ensure the establishment of attachment with an appropriate biologic width [16].

The study by Berglundh and Lindhe, 1996, confirmed those statements and revealed that implant soft tissues consisted of a junction epithelium that was about 2 mm and connective tissue attachment approximately 1 mm [17]. At experimental sites where the ridge mucosa prior to abutment connection was less than 2 mm, wound healing consistently included bone resorption [17]. This implied that a certain minimum width of the peri-implant mucosa about 3 mm might be required, and that bone resorption might take place to allow a stable soft tissue attachment to form.

Attachment of Peri-implant Tissue to an Abutment

The attachments between soft tissues and implant abutments contain complex structures called hemidesmosomes [18, 19], which have a definite role in providing specific signal transduction, and participate in regulation of cell proliferation and differentiation [20]. Such functions might be altered when tissue contacts with different implant materials or different surface topographies [2].

Listgarten, 1996, analyzed the intact interface between soft connective tissue and titanium-coated epoxy resin implants, and reported that parallel orientations of collagen fibers to the titanium layers were observed with no inserted layers. The attachment of the connective tissue to the transmucosal portion of an implant was regarded as being weaker than soft tissue attachment to the surface of a cementum [21]. Therefore, improving the quality of the soft tissue to implant interface is considered to be important.

Animal Histological Studies

Due to ethical considerations of the histological studies, previous researches, which designed to compare effect of different materials in vivo, were performed in animal models.

Studies by Abrahamsson, et al, 1988, and Welander et al, 2008, which observed the soft tissue healing, found that the conditions of soft tissue forming around gold alloy abutments were poorly attached to the abutments compared with titanium and zirconia abutments in dog models [8, 10]. In contrast, the study which included the implants and the surrounding tissue [9], revealed osteointegration was achieved in surfaces made of both titanium and gold alloy and the peri-implant soft tissue dimensions were not different in both materials [9]. Moreover, Kohal et al, 2004 demonstrated the same peri-implant soft tissue dimensions around titanium and zirconia implants installed in the monkeys [22].

Human Histological Studies

To date, there were a few studies in human, which compared the response of soft tissue around different types of abutment materials. The previous methods in those studies did not illustrate direct relationship of soft tissue levels to the abutments. Those studies were, however, compared between two materials and only one study, which compared the inflammatory response by using immunohistochemical staining to detect the differences in cell responses.

Van Brakel, et al, 2012, compared the soft tissue response of two different abutment materials, titanium and zirconia, in the split mouth designed of 17 patients. After 3 months, tissue collections were performed and the results showed very little sign of inflammation in the specimens of both materials. There was no statistically significant difference in the micro vascular density (MVD) and inflammation grading score between titanium and zirconia abutment [13].

Degidi, et al, 2006 compared the soft tissue response to titanium and zirconium healing abutments and gingival biopsies showed higher level of the inflammatory infiltrate in the titanium specimens. Higher values of MVD were observed in the titanium specimens compared to zirconium oxide abutments. Immunohistological markers indicated that titanium abutments had higher bacterial accumulation than zirconia samples [12].

From the studies revealed that the tissues around titanium healing abutments in human might undergo equal or higher rate of inflammation processes compared to the inflammation processes observed around zirconium oxide abutment.

Histological Processes

To study the histomorphometric of implant abutments in humans, there are some technical difficulties in preparing the specimens, which consisted of different degrees of hardness; metal and soft tissue. To overcome such difficulty of cutting through different hardness, several methods were performed to remove the implant before sectioning, such as, fracture technique, mechanical separation after embedment, and cryofracturing technique [23]. The fracture technique, in which an implant is removed during decalcification with ethylenediaminetetraacetic acid (EDTA) before embedment, has been used frequently to observe the histology of soft tissue in both animal and human models [8, 16, 17, 24]. However, the disadvantage of the technique was that the attachment between the peri-implant tissues might be altered [23].

Donath and Breuner, 1982, have introduced a slide preparation method using resin embedding technique. This technique enables sectioning through a hard material without the need of tissue separation [25]. A resin embedding technique, which resulted in implants retained in the sections, usually provides observers illustrations of relationships between implants and histological landmarks such as gingival margin, apical part of epithelial cells and the most coronal bone to implant contact [23]. However, this technique can be very sensitive in performing very thin sections to observe under light microscope [26]. A special designed cutting and grinding machine was suggested to control the grinding pressure, and provided uniform cutting surfaces of specimens [23]. And the sections need to be further polished with polishing machine to allow even surfaces of the ground sections [23].

Recently, Schwarz, et al, 2013, has used this technique to evaluate the fiber orientation and histomorphology of human soft tissue on modified titanium surface abutments [27]. The study reported that this technique can illustrate the relationship of soft tissues around the experimental abutments and allowed direct measurements of the distance between histological landmarks, relatively to the length of abutments. This remark was also based on special design tool for tissue harvesting protocol.

Healing of Peri-implant Soft Tissue

The soft tissue around implant abutment underwent healing processes after the implants were placed in the jaws. Immediately after implant placement, blood clots separated the oral mucosa from the implant surface. The inflammatory cells, primarily polymorphonuclear cells, infiltrated to the area. A blood clots were replaced by dense fibrin networks. Fibroblasts then invaded the fibrin network and produced collagen fibers to form a connective tissue. Two weeks following implantation, newly formed connective tissue contained numbers of vascular units and fibroblasts were in close contact with the implant surfaces [11]. Proliferation and migration of epithelial cells occurred around 1–2 weeks of healing periods and leaded to the formation of a junctional epithelium, which in turn lengthened the interface between the implant surface and the peri-implant mucosa [11]. The apical migration of the peri-implant junctional epithelium was completed between 6 and 8 weeks and the fibroblasts formed a dense layer over the titanium surface at that time. Maturation of the peri-implant mucosa occurred between 6 and 12 weeks following implant placement and was mainly characterized by a mature epithelial barrier and collagen fibers [11], with few blood vessels and paralleling alignment of collagen fibers. The healing time was reported to require at least 6 weeks in Labrador dogs.

A new human model was introduced by Tomasi et al, 2013. They investigated the morphogenesis of the peri-implant mucosa during the first 12 weeks of healing and observed that a soft tissue barrier adjacent to titanium implants developed completely within 8 weeks.

While the soft tissue seal around teeth develops during tooth eruption, the peri-implant mucosa forms after the creation of wounds in soft and hard tissues. The wound healing phase may occur following the closure of a mucoperiosteal flap around the neck portion of an implant. Since wound healing occurs in the presence of biomaterials, adaptations of the soft tissue to this biomaterial have to be taken into consideration.

Histomorphometrical Analysis and Tissue to Abutment Measurements

In many studies, the landmarks were identified in the histological sections, such as, abutment shoulders, marginal portion of the peri-implant mucosa, apical extension of the long junctional epithelium, the apical aspect of the subepithelial connective tissue, and measurements were made by creating vertical lines following the long axis of the implants. The vertical distances between the landmarks were determined [8, 10, 13, 17, 24, 27].

Schwarz, et al, 2013 used the referent points on peri-implant tissue and on abutment to measure the distance and calculate percentage of soft tissue to abutment contact comparing 3 different abutments [27].

Inflammatory Infiltrated cells Measurement

The observations of inflammatory infiltration have been used to study the characteristic of the inflammatory cells in peri-implant tissue. Observation could be scored to describe inflammatory response in term of density, intensity, and location [12, 13, 23, 28, 29].

From previous study, inflammatory infiltrations which were rich in leukocytes, were observed in peri-implant tissue as a result of immunological respond against oral bacteria [29]. Previous immunohistological study described differences amount of B cells and plasma cells dominated in the infiltrate area of failing implant [30].

Pongnarisorn et al, 2006, observed association of inflammatory infiltrates and implant surfaces, using criteria to grade the density of inflammatory cells. The results revealed all peri-implant tissues surrounding tested implants exhibited some degree of inflammatory infiltrate. The inflammatory infiltrates were demonstrated in the connective tissue of the peri-implant mucosa immediately beneath the epithelium and in perivascular areas deeper in the tissue. The subepithelial areas had a higher density of inflammatory cells. While the density of cells at the perivascular areas were lesser [29].

In van Brakel, 2012, study, inflammations were graded into 4 scores, depended on inflammatory infiltrate and fibroblast cells [13]. Other studies Inflammatory infiltrations were measured as scores depending on the intensity of the inflammatory cells presented in the tissue sections [12, 13, 23, 29].

CHAPTER 3

MATERIALS AND METHODS

Research Design

A double blinded, randomized clinical trial was designed to compare differences of soft tissue responses around 3 types of abutment materials.

Diagram of Study Design

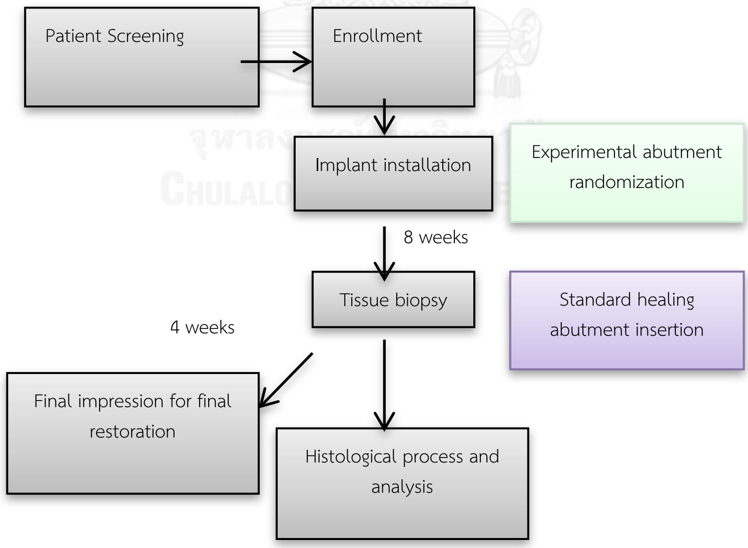


Figure 2 Diagram of the study design

Ethical Considerations

We considered according to Belmont principal all patients included were agreed to participate in this study, with their signatures on the consent forms. Participants were in closed follow up and were randomly assigned to the abutment groups. The method of the study, which involved soft tissue collection using surgical blade, had been approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand. The study approval number was HRE-DCU 2014-051.

Population and Sample

Patients who had posterior teeth extraction at least 4 months, adequate bone quantity at the experimental site for the insertion of 4.5 or 5.0 mm diameter implants, sufficient band of keratinized mucosa (>5 mm) and agreed to participate in the study, were included. The exclusion criterions were patients who were smokers, had systemic diseases requiring routine use of antibiotics, and were pregnant.

This study was designed to use descriptive statistic due to the method to measure the histomorphometric in this study was new, and there has been no research which use this method to study soft tissue response of different abutment materials in human.

The number of sample size in this study was designed according to the previous study [24], which conducted the histomorphometric measurements. The study suggested 5 subjects per group.

In this study, 15 participants were included and randomly assign to 3 groups, 5 of each.

Allocation Technique

Patients were allocated to one of the three treatment groups: group1=titanium, group2=zirconia, group3=gold alloy. Before delivering of study abutments, a randomization was performed by having a patient choose an envelope. Each envelope was opened right after an implant fixture insertion to blind an operator. Then, experimental abutments were screwed on top of the implant fixtures on the surgery day, according to the type of material written in the selected envelope.

Experimental Abutment

Group1: The TiDesign™ abutment, diameter 5.5mm x3mm (product code 24236, Astra Tech Dental, Densply, Mölndal, Sweden) (Figure 3a)

Group2: The ZirDesign™ abutment, diameter 5.5mm x3mm (product code 24708, Astra Tech Dental, Densply, Mölndal, Sweden) (Figure 3b)

Group3: The CastDesign™ abutment diameter 4.5mm (product code 22844, Astra Tech Dental, Densply, Mölndal, Sweden) (Figure 3c)

a b c

Figure 3 The illustrations of prefabricated abutments, (a) group 1 = TiDesign™ abutment, (b) group 2 = ZirDesign™ abutment, (c) group 3 = CastDesign™ abutment

Intervention

Surgical protocol

Titanium implant fixtures from Astra Tech Dental Company implants, OsseoSpeed^{TM} (Densply, Mölndal, Sweden), diameter 4.5 and 5.0, and length 9 mm and 11 mm, were selected depending on each individual clinical and radiographic evaluation. Surgical protocol was performed with a standard procedure under local anesthesia by the dentists who studied at Esthetic Restorative and Implant Dentistry program, Chulalongkorn University, during years 2014-2015, under a supervision of one experienced surgeon. A crestal incision was performed at the center of the planned implant fixture. Then, the flap was operated to expose the surgical site. The implant site was prepared and implant fixture was positioned at the crestal bone level in all aspects.

An abutment, according to the type written in the selected envelope, was positioned on implant fixture instead of routine using of healing abutment. The flaps was approximated and sutured. The occlusal part of the abutment was reduced, to avoid any contact with the opposing teeth in any direction. Then, the hole of the abutment was screwed and covered with esthetic tape and resin composite.

Every patient was prescribed an antibiotic for 1 week interval and a 0.2% Chlorhexidine month rinse for 2 weeks. Two weeks post-operation, the patients were asked to come back for wound evaluation and stitch removal. Then, at 8 weeks, patients were appointed for a tissue collection visit.

Specimen biopsy

Eight weeks after implant placement, clinical condition of soft tissue at left side and right side of the abutment was evaluated by one calibrated examiner, in termed of color vise using the Gingival Index (GI) Criteria [31], with no probing, as to probe would cause destruction to the attachment (Figure 4). The criteria were described in Table 1.

Figure 4 The clinical picture demonstrates gingival conditions around the abutment at 8 weeks after implant surgery

Table 1 Description of GI index (Löe and Silness, 1963)

Score	Description
0	Pale pink to pink
1	Slightly more reddish or bluish-red
2	Red or reddish-blue
3	Markedly red or reddish-blue and enlarged, Ulceration

Table 2 Description of surgical score

Score	Description
1	no detachment, firm tissue
2	some detachment, firm tissue
3	full detachment, loose tissue

The peri-implant tissue biopsy was carried out by one examiner using surgical blade no. 12D and 15C. The tissue was carefully cut in a circular shape approximately 1 mm away from the outer surface of the abutments. The angle of the blade was paralleled to the outer surface of the abutment (Figure 5), which resulted in a ring shape of soft tissue attached to the abutment. Then, the abutment was unscrewed and gently removed together with attached peri-implant tissue (Figure 6). During the tissue harvesting process examiner observation was also recorded as surgical score, by focusing on the consistency of soft tissue; firm or loose, and the harvesting procedure; attached or detached. The surgical score was described in Table 2.

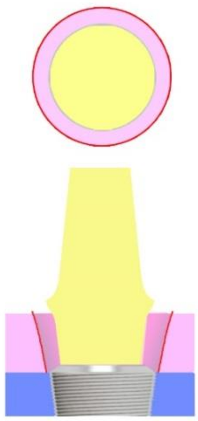


Figure 5 The red lines illustrate the positions and angulations of the surgical blade

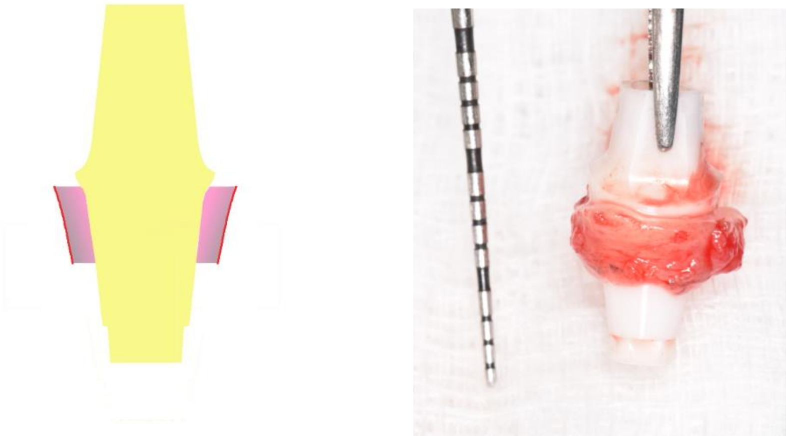


Figure 6 The illustration and the clinical picture of the biopsy tissue

After the tissue biopsy, regular titanium healing abutment, diameter 6.5 mm, was placed on an implant fixture. One month later, the patients were appointed for an impression visit for prosthetic constructions.

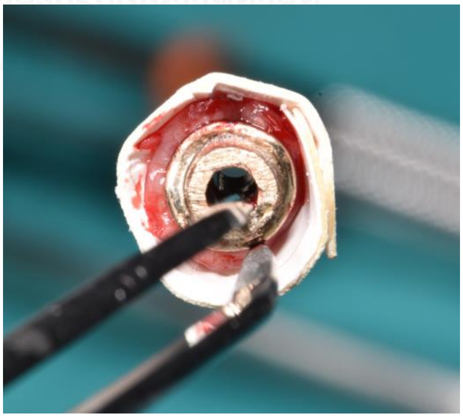


Figure 7 The clinical picture demonstrates the use of thick paper for holding the biopsy specimen before fixation

Specimen Preparation

After biopsy, a piece of thick paper was used to hold each specimen in its place (Figure 7). Specimen was fixed with 10% formalin for 1 day and underwent serial dehydration procedures with 70%-100% ethyl alcohol for 1-week duration. The specimen was then infiltrated with resin (Technovit 7200 VLC, Kultzer, Friedrichdorf, Germany): ethyl alcohol, 1:1, 3:1, and pure resin, each concentration taking 3 days. Afterward, the specimen was placed in a plastic block. The mid buccal aspect of the abutment was faced down toward plastic block and the block was filled up with resin and light cured for 12 hours. The resin block was mounted with mixing powder and liquid of Technovit 4000 VLC (mounting back slide). The front slide was mounted with light cure resin (Technovit 7210 VLC, Heraeus Kultzer, Wehrheim, Germany), and then the back slide was placed on the machine holder (Exakt® Apparatebau, Norderstedt, Germany) (Figure 8). The cutting blade was set as 250 microns from the front slide. The block was cut, and remounted with the next front slide (Figure 9-10), finally, resulted in 5-6 slides of the specimen from each block. The slides with the specimen attached were then grinded with Exakt Microgriding Machine, using silicon carbide papers no. 800 and 1200 and 1800 for titanium and gold alloy specimens and diamond sand paper no. 800, 1200 and 1800 for zirconia specimens, to the specimen thickness of 40-60 micron. Then, slides were polished with silicone carbide polishing paper no. 4000.

All specimens were stained with Haematoxylin and Eosin (H&E). Briefly, the sections were rinsed in distilled water for 30 minutes, stained for 30 minutes with Harris Hematoxylin (Leica Biosystems, Richmond, IL, United states), rinsed in tap water for 10 minutes, stained with Alcoholic Eosin Y 515 (Leica Biosystems, Richmond, IL, United states), dehydrated in graded ethanol and mounted with Sub-X Mounting Media (Leica Biosystems, Richmond, IL, United states).

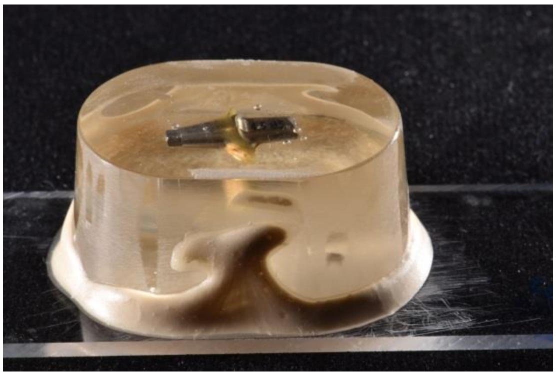


Figure 8 The picture of the specimen, embedded in the resin block, and glued to the back slide (mounting back slide)

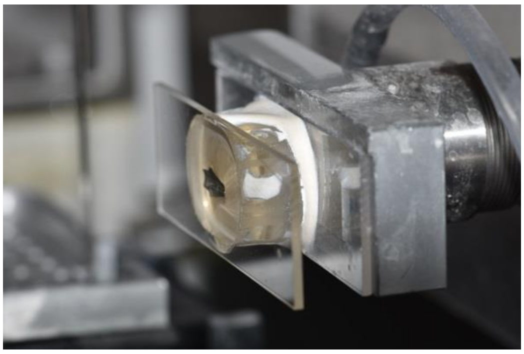


Figure 9 The picture of the specimen with the front mounted to the resin block, and the back slide placed on the holder of the cutting machine

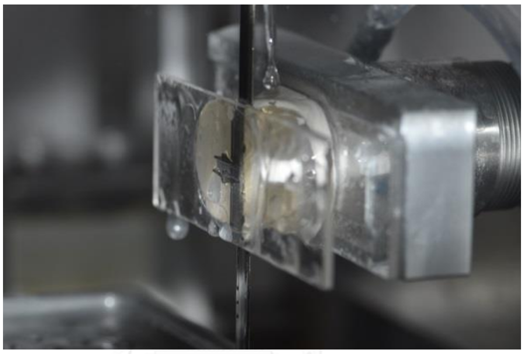


Figure 10 The picture of the cutting process with water irrigation

Histological Evaluations

Histological evaluations of specimens were conducted by one blinded experience pathologist using light microscope (Olympus, BX53, Tokyo, japan), magnification of 20X, 40X, and 200X. Prior to the measurement, a calibration procedure was initiated, and revealed that repeated measurement of 6 different histological slides were similar at >95% interval. To histologically evaluate the peri-implant attachment and inflammatory infiltration, two most central slides from each specimen, were picked up for evaluation and total of four observations per patients were used for statistical calculations (2 slides x 2 sides).

Histological attachment percentage

Measurements were performed at magnification of 40x under light microscope (Olympus, BX53, Tokyo, japan) attached with digital camera (Olympus DP21, Tokyo, Japan). Pictures were captured. Then the measurements were made by creating lines parallel to the surface of the implant abutments. The total attachment length was measured from the most coronal part of epithelium attachment to the most apical part of epithelial or connective tissue (Figure11). The areas which gaps were presented were measured and attachment percentage was calculated by total attachment length minus total gap length divided by total attachment length. The measurements were conducted twice. Repeated measurements were performed one week after, and then the percentages were averaged.

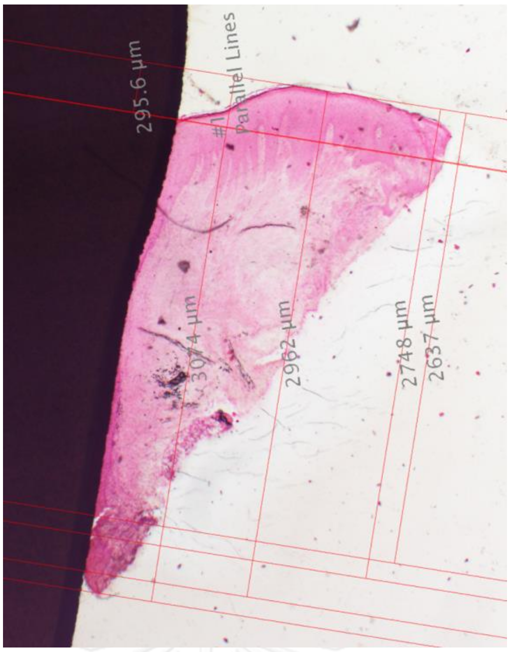


Figure 11 The picture represents the evaluation of attachment percentage by drawing parallel lines to the abutment surface

Inflammatory extent grade

The extensions of inflammatory cells were scored using semi quantitative scale (Table 3). The scores were graded according to the amount and location of the inflammatory cells presented in each specimen (Figure 12).

Inflammatory cellularity grade

A 3-grade system of inflammatory cell density was evaluated (Table 4) using the criteria described by a previous study **[29].** Two pictures from each side, captured at region of interest (ROI) at left and right sides of the abutment interface, using high magnification of 400X, were analyzed and the maximum score of each side was used for calculation. All pictures were captured by a digital camera (Olympus DP21, Tokyo, Japan) (Figure 13).

Table 3 Inflammatory extent grade and description

Grade	Description
1	Inflammatory cells at sub epithelium (invasion <30% of the tissue)
2	Inflammatory cells extend to inner connective tissue (Invasion >30% of the tissue)
3	Generalize inflammatory infiltrate (>50% of total tissue of the tissue)

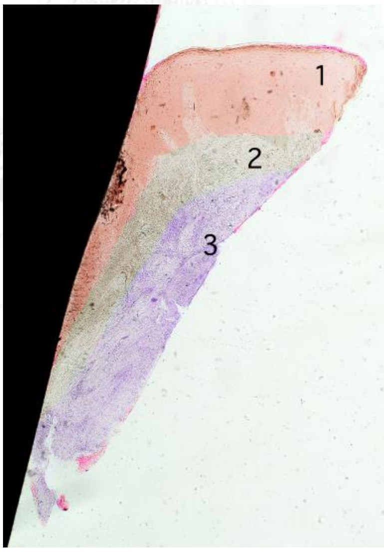
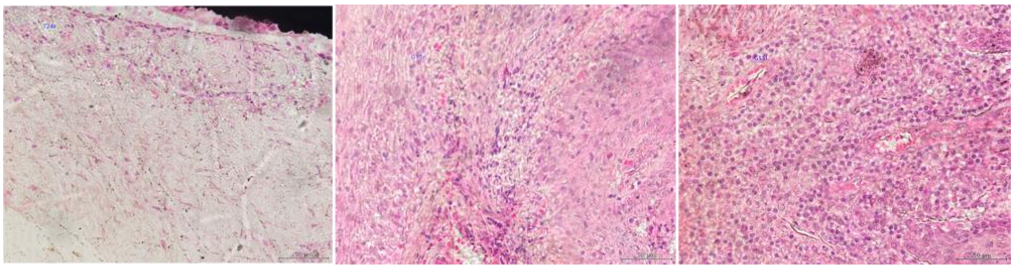


Figure 12 The Illustration of areas of inflammatory extent grade; grade1 = red, grade2 = green, grade3 = purple

Table 4 Inflammatory cellularity grade and description

Grade	Description

1	Sparsely infiltrating cells
2	Moderately infiltrating cells
3	Densely infiltrating cells



a b c

Figure 13 Pictures of ROI; a. represents inflammatory cellularity grade1, b. represents inflammatory cellularity grade2, c. represents inflammatory cellularity grade3

DATA ANALYSIS

First, descriptive statistics were used to describe the data as proportion for ordinal scale variables and the mean and standard deviation for a continuous variable.

Then, data were further analyzed using statistical models depended on the measurement scale of the variables (in our case: ordinal or quantitative), and whether multiple observations were taken from the same patient (i.e.; a clustering effect).

The outcome variable, GI, had two observations per patient (both sides of the implant observed), but only the maximum value from each patient was chosen. As only scores of 0 or 1 were observed in all our patients, we conducted a Chi-Square test to examine whether the distribution of GI was independent from the treatment group.

The outcome variable, a surgical score, was measures on an ordinal scale, with a single observation per patient. The hypothesis of group equality was conducted using the Kruskal-Wallis test, followed by Mann-Whitney U test with Bonferroni correction for pairwise comparisons.

For the continuous outcomes, attachment percentage, had four observations per patient. To account for the within subject correlation among these observation and preset appropriate confidence intervals, we employed Linear Mixed Modeling (LMM).

Finally, the outcome inflammatory extent grade and inflammatory cellularity grade were ordinal outcomes, which also had four observations per patient, were analyzed using the proportional odds ordinal logistic mixed effect regression.

All statistical analysis was conducted using the R statistical language (V 3.3.0; R Core Team, 2016). Linear mixed modeling was performed using the lme4 R library (Bates, 2016), and the ordinal logistic mixed effect regression, using the ordinal library (Christensen, R. H. B., 2015). A significance level of 0.05 was used throughout all analysis.

CHAPTER 4
RESULTS

A total of 15 edentulous spaces in 10 healthy patients, 6 males and 4 females were analyzed. Patients who had two edentulous areas were asked to choose the envelopes twice and were given two abutments as stated in the envelopes. Five patients who received two abutments were marked with letters a, b, c, d, and e (Table 5-8).

Mean age was 52.8 for the titanium group, 55.6 for the zirconia group, and 56.6 for gold alloy group. Three implants with diameter 4.5 was placed in second premolars and 12 implants diameter 5.0 were place in first and second molar areas.

The sex, age and tooth number of each experimental groups were clarified in the Table 5-8.

All implants healed completely without any complication. However, during the attempt to remove the abutment from implant fixture, in one case of the gold alloy abutment group, the abutment fell out of the forcep and the patient accidentally swallowed the abutment, therefore, the case had to be excluded, and a new patient was recruited. Follow up of the patient was well.

Table 5 Demographic data of cases in a titanium group

Case		Sex	Age	Tooth number
T1	(a)	F	57	36
T2	(b)	F	61	25
T3		M	41	36
T4	(d)	M	48	37
T5	(e)	M	57	36

Table 6 Demographic data of cases in a zirconia group

Case		Sex	Age	Tooth number
Z1	(a)	F	57	25
Z2	(b)	F	61	36
Z3	(c))	F	60	36
Z4		F	40	36
Z5		M	60	46

Table 7 Demographic data of cases in a gold alloy group

Case		Sex	Age	Tooth number
G1		F	62	36
G2	(c)	F	60	46
G3	(e)	M	57	45
G4		M	55	47
G5	(d)	M	48	46

Table 8 Tooth numbers and abutment types of five patients received two abutments.

Patient	Tooth number	Abutment type	Tooth number	Abutment type
a	25	Titanium	36	Zirconia
b	36	Titanium	25	Zirconia
c	36	Zirconia	46	Gold
d	37	Titanium	46	Gold
e	36	Titanium	45	Gold

Attachment evaluation

Evaluations of the attachment were described by 3 variables; maximum GI score, surgical score and attachment percentage (Table 9).

On one case of titanium group (T5), the data from 2 histological slides had to be excluded, due to the slide defects, which prevented observer from evaluating the results.

In titanium group, three samples had GI of left and right side = 1, two samples had GI of left and right side = 0. Surgical score revealed only one sample which the tissue still attached to the abutment during removal of the abutment from the fixture, given score of 1. Three samples presented some detachment but firm tissue condition, given score of 2. In one sample, tissue was very loose tissue and fully detached from the abutment while removing from implant fixture and was not be able to fix back in its place, given score of 3. The percentage of the attachments in histological slides were 76.17%-85.24.% and the mean was 80.80(4.10) (Table 9).

In zirconia group, two samples had GI of the left and right side = 0. One sample had GI of left and right side = 1, one sample had GI of left =1 and right=0, and one sample had GI of left =0 and right=1. Surgical score 1 was given to one sample with the tissue still attached firmly to the abutment. Other four samples presented some detachment, given score of 2. The percentages of attachments were 59.87%-92.06%, mean was 80.12(12.06) (Table 9).

In gold alloy group, all samples had GI of the left and right side = 1. Surgical attempt to harvest the soft tissue revealed three samples presented some detachment but firm tissue condition, given score of 2. In two samples, tissue was very loose and fully detached from the abutment, given score of 3. The percentages of attachments were 38.80%-76.39%, mean was 50.66(16.16) (Table 9).

Regarding the GI score, titanium and zirconia demonstrated the same results, while gold alloy received GI score of 1. The gingival tissue presented slightly red, in all sample. For surgical score zirconia had a better result with 0% of score 3, while 40% of the case in gold alloy group received score 3. For mean attachment percentage, titanium and zirconia exhibited almost similar mean attachment percentage while gold alloy received much lower percentage of 54.66% (Table 10).

By further analyzing the data, chi-square test suggested no evidence of association between maximum GI score and abutment types (p-value = 0.071), and Kruskal Wallis test suggested no statistically significant differences of surgical score among groups (p-value= 0.262). The LMM demonstrated that abutment type had a significant effect on attachment percentage (p-value = 0.004). Post-hoc comparison demonstrated that gold alloy abutment resulted in a significant reduction in attachment percentage, as the mean went down by -0.262. However, zirconia had very little effect which the mean was -0.007 lower than titanium group.

Table 9 Data on gingival index of left side (GI(L)) and right side (GI(R)), surgical observation and attachment percentage (%Attachment) in histological slides of each cases

Case	GI(L)	GI(R)	Surgical observation	%Attachment
T1	1	1	Some detachment, Firm tissue 2	85.24
T2	0	0	No detachment, Firm tissue 1	83.08
T3	0	0	Some detachment, Firm tissue 2	78.73
T4	1	1	Full detachment, Loose tissue 3	76.17
T5	1	1	Some detachment, Firm tissue 2	-
Z1	1	1	Some detachment, Firm tissue 2	59.87
Z2	0	0	Some detachment, Firm tissue 2	83.66
Z3	1	0	Some detachment, Firm tissue 2	84.02
Z4	0	1	No detachment, Firm tissue 1	92.06
Z5	0	0	Some detachment, Firm tissue 2	81.02
G1	1	1	Full detachment, Loose tissue 3	38.80
G2	1	1	Some detachment, Firm tissue 2	66.97
G3	1	1	Some detachment, Firm tissue 2	76.39
G4	1	1	Some detachment, Firm tissue 2	44.19
G5	1	1	Full detachment, Loose tissue 3	46.92

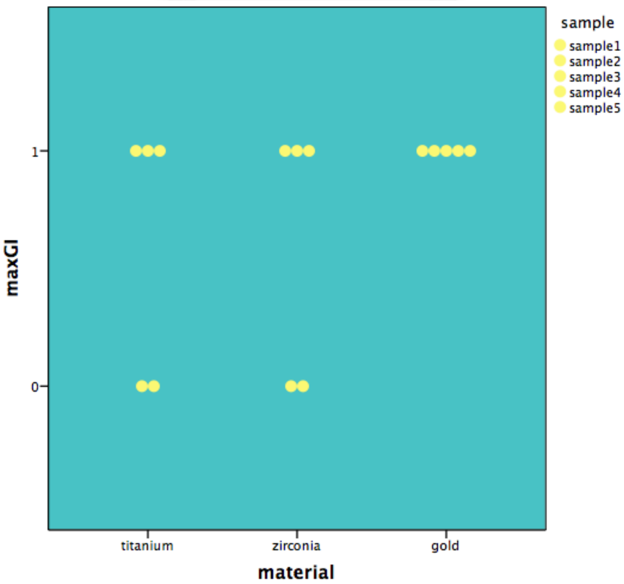


Figure 14 The dot-plot diagram of maximum GI score of each materials

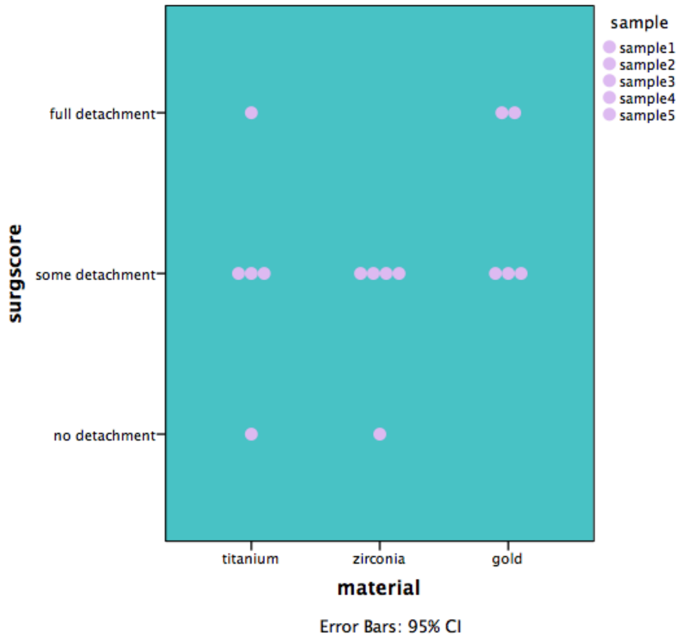


Figure 15 The dot-plot diagram of surgical score of each materials

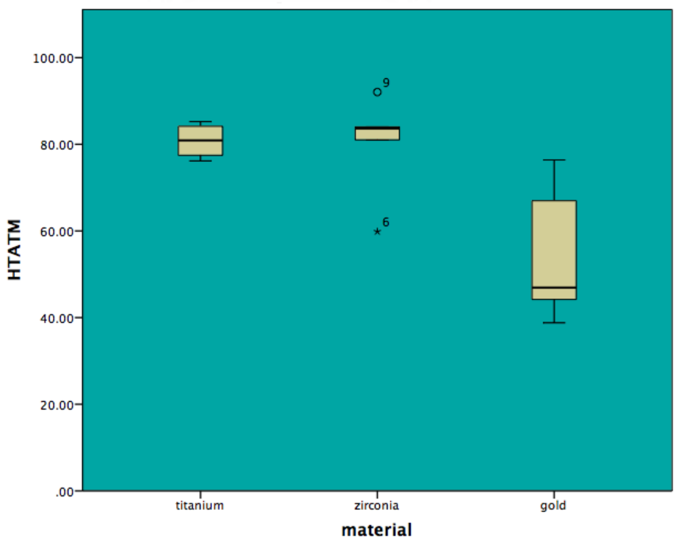


Figure 16 The box-plot diagram of total attachment percentage of each materials

Table 10 Descriptive results of maximum GI score (Max.GI Score), surgical score, attachment percentage (% Attachment), inflammatory extent (Inf. Extent) grade, and inflammatory cellularity (Inf. Cell) grade in all cases

Abutment	Max.GI Score		Surgical Score			% Attachment	Inf. Extent			Inf. Cell		
	0	1	1	2	3	Mean(SD)	1	2	3	1	2	3
Titanium	40%	60%	20%	60%	20%	80.80(4.10)*	37.5%	50%	12.5%	43.8%	56.3%	0%
Zirconia	40%	60%	20%	80%	0%	80.12(12.06)	40%	50%	10%	15%	75%	10%
Gold alloy	0%	100%	0%	60%	40%	54.66(16.16)	16.7%	55.6%	27.8%	22.2%	44.4%	33.3%

Table 11 Data on maximum inflammatory extent grade at left side (Max.Inf.Extent (L)) and (Max.Inf.Extent (R)) and maximum inflammatory cellularity grade at left side (Max.Inf.Cell (L)) and right side (Max.Inf.Cell (R)) of each cases

Case	Max.Inf.Extent (L)	Max.Inf.Extent (R)	Max.Inf.Cell (L)	Max.Inf.Cell (R)
T1	1	1	2	1
T2	2	3	2	2
T3	3	2	2	2
T4	2	2	1	1
T5	-	-	-	-
Z1	1	1	2	2
Z2	2	2	2	2
Z3	2	3	3	3
Z4	2	2	2	2
Z5	1	2	2	2
G1	3	3	3	3
G2	3	3	3	3
G3	2	2	2	2
G4	2	2	2	2
G5	2	2	2	2

Inflammatory Response Evaluation

Evaluation of the inflammatory infiltrate in histological slide was reported in inflammatory extent grade and inflammatory cellularity grade.

There were defects on two slides of the sample T5 and one slide of the sample G5, which prevented evaluator to detect inflammatory cells. Therefore, those slides were excluded.

In titanium group, the maximum inflammatory extent grades of the left side of abutments were 1,2,3,2,- and right side were 1,3,2,2,-. The maximum inflammatory cellularity grades of the left side were 2,2,2,1,- and right side of abutments were 1,2,2,1,- (Table 11).

In zirconia group, the maximum inflammatory extent grades of the left side of abutments were 1,2,2,2,1 and the right side of abutments were 1,2,3,2,2. The maximum inflammatory cellularity grades of the left and right side of abutment were 2,2,3,2,2 (Table 11).

In gold alloy group, the maximum inflammatory extent grades of the left side and right side of abutments were 3,3,2,2,2. The maximum inflammatory cellularity grades of the left and right sides of abutment were 3,3,2,3,3 (Table 11).

For Evaluation of the inflammatory extent grade, the grade 3 represents the worst condition and the gold alloy presented the highest percentage in grade 3, with more than twice the percentage compared to those of the other two groups (Table 10).

For inflammatory cellularity grade, the highest percentage of specimen presented by grade3 was found in gold alloy (33.3%). The highest percentage of specimen presented by grade2 was found in zirconia (75%). And the highest percentage of specimen presented by grade1 was found in titanium (43.8%) (Table 10).

Further analysis of the data, using ordinal logistic mixed effect regression models, found no statistically significant differences for both variables. But for inflammatory extent grade, the odds of being one grade higher for zirconia abutment group was 0.87 times and the odds of being one grade higher for gold alloy abutment group was 5.18 times that of titanium abutments. Moreover, the inflammatory cellularity grade both abutment, zirconia and gold alloy, leaded to a substantial increase in the odds of being in a higher count group with 7.5 and 17.8 times, respectively.

Table 12. Descriptive results of five cases that patients received two types of abutment by abutment type, tooth number, maximum GI (MAX. GI) score, surgical score, attachment percentage (% Attachment), maximum inflammatory extent grade of left side (MaxinfE(L)) and right side (MaxinfE(R)), and inflammatory cellularity grade of left side (MaxinfC(L)) and right side (MaxinfC(R))

Case	Material	Tooth	MAX. GI	Surgical score	% Attachment	Maxinf E(L)	Maxinf E(R)	Maxinf C(L)	Maxinf C(R)
a	Titanium	25	1	2	85.24	1	1	2	1
	Zirconia	36	1	2	59.87	1	1	2	2
b	Titanium	36	0	1	83.08	2	3	2	2
	Zirconia	25	0	1	83.66	2	2	2	2
c	Zirconia	36	1	2	84.02	2	3	3	3
	Gold alloy	46	1	2	66.97	3	3	3	3
d	Titanium	37	1	3	76.17	1	1	2	2
	Gold alloy	46	1	3	46.92	2	2	2	2
e	Titanium	36	1	2	-	-	-	-	-
	Gold alloy	45	1	2	44.19	2	3	3	2

When comparing data of the patients who received two types of abutments (Table 12): titanium vs. gold alloy abutments (case d, e) or zirconia vs. gold alloy abutment (case c), we found that the maximum GI score and surgical score were similar for both abutments. However, the attachment percentages were detected lower in gold alloy abutments, while maximum inflammatory extent grade were lower in zirconia and titanium abutments compared to gold alloy.

Comparing titanium and zirconia, the results showed that titanium had better attachment percentage in one case (case a), but lower in the other case (case b). The inflammatory extend grade were recorded higher in one side of titanium in case b and the maximum inflammatory cellularity grade was higher in one side of zirconia.

These comparisons reported the same results with the LMM and ordinal logistic mixed effect regression model.

CHAPTER 5
DISCUSSION AND CONCLUSION

Discussion

Histological evaluation of attachment in this study resulted from both factors, the attachment formed toward material, and specimen handling and processing. Therefore, the attachment percentage was not the real attachment value that presented clinically, but it represented the histological evidence resulting from this experiment. To control the specimen handling technique, one investigator performed all tissue harvesting procedures in all groups with the same technique by placing the forcep at flat buccal side of abutments and pulling the abutment and tissue out together. The most detached area occurred at lingual and peri-implant tissue was positioned to its place with the paper cap. However, the areas that observed in the histological slide were at the proximal areas, which tissue depositions occurred less. And we used 2 central slides, given 4 observation spots at proximal areas, for calculation. Good surgical skills and experiences in the harvesting procedure were essential to maintain the in-vivo integrity of the peri-implant tissue. Moreover, we recommended using a thick paper to wrap the harvested specimen before put in the 10% formalin fixative to help keeping the attachment.

The technique used to embed a specimen in resin, had many critical steps to be concerned [23]. Specimen fixation, dehydration, and infiltration steps required at least 3 weeks depending on specimen thicknesses. Inadequate time resulted in black burning areas in a histological slide. Voids and gaps could occur while pouring light cure resin into block holding specimens, and could cause defects in histological slides. To avoid that, slow and continuous pouring of the resin in a block is recommended to reduce chances of air trapped. In this study, two slides from the titanium group and one slide of the gold alloy group had to be excluded due to voids presented in the middle of the tissue. Moreover, different slide thicknesses for resin embedded specimens, ranging from 10-120 micron, were reported in previous studies [8, 24, 27, 32]. The thicknesses of the slides which were suitable for evaluations in this study were 40-60 micron. The slides that were too thin caused dislodgement of the metal part and resulted in crack lines on the remaining part of the specimens. This occurred on one slide of titanium group, which was excluded. Samples, prepared too thick, had several focusing depth under light microscopy, which prevented the observer from inspecting the cell morphology. Thus, operator experiences were needed to obtain satisfactory results.

Findings from this study demonstrated that a gold alloy abutment group resulted in different clinical observations of GI compared to titanium and zirconia group. The tissue around titanium and zirconia abutments presented normal to slightly red color, while, the tissue around gold alloy abutment was found to be only slightly red. Additionally, one case, gold abutment accidentally fell out and the patient swallowed the abutment. Therefore, the case was excluded. The highest percent of detachment cases observed by surgical score was found in gold alloy group. And the histological attachment percentage confirmed the significant results that gold alloy group had significant lower percentage of tissue attachment compared with the other 2 groups (p-value =0.004).

Our results that the gold alloy group was inferior to the other abutment materials was in agreement with the previous animal study [8], which found significant gingival recession and marginal bone destruction observed at 5 months healing in the gold alloy group, but at titanium and zirconia groups were stable.

Likewise, an animal experiment by Abrahamson, et al., 1988 reported that soft tissue dimensions at sites with gold abutments were smaller after 6 months healing period [10]. Moreover, the connective tissue interfaces at gold alloy abutments contained lower amounts of collagen and fibroblasts and larger fractions of leukocytes than the connective tissue interface of titanium and zirconia abutments. In our study, the same trends were reported, as the odds of inflammatory extension and density of infiltrate cells were observed to be higher in the gold alloys group as well.

On the other side, animal study by Abrahamson & Cardaropoli, 2007 revealed a similar dimension of the barrier epithelium and the position of the marginal bone at titanium and gold-alloy implants at healing time of 6 months [9]. The absence of differences in soft tissue dimensions was observed at 2 months of healing in the study of Welander et al., 2008. In our study, there was no statistically significant difference of the total length of soft tissue between three abutment groups, which could mean the dimension of soft tissue healing was comparable among three materials at two months healing.

Only Vigolo, et al, 2006, conducted 4 years follow up of titanium and gold-alloy abutments and found no significant different behaviors of peri-implant marginal bone and of peri-implant soft tissue level when titanium abutments or gold-alloy abutments were used [14]. With the limitation of our study, the results reported the conditions of tissue after 2 months of implant surgery. The results represented early maturation periods of the soft tissue healing toward abutment materials which demonstrated less resistance of attachment and higher trend of inflammatory responds in the gold alloy abutment group.

Comparing titanium and zirconia abutments, in this study, no difference in terms of clinical sign of inflammation, surgical score, tissue attachment to abutment material, and inflammatory responses were found. However, when using analytical models, the odds ratio of inflammatory extent grade was lower in zirconia but the odds ratio of inflammatory cellularity grade in zirconia group was 7.598 times higher compared to that of the titanium specimens. This could explain the characteristics of inflammatory cells detected between titanium and zirconia abutments, but more samples should be included to confirm the results.

The study by van Brekel 2012 reported no statistically significant difference of the inflammatory grade scale and microvascular density observed in 17 patients who had received both titanium and zirconia abutments, the study had low power and suggest 3000 patient to be included [13]. Degidi, et al, 2006 found no statistical significant of inflammatory cells among titanium and zirconia abutments. However, higher values of micro vascular density, vascular endothelial growth factor, and higher Ki-67 antigen expressions were observed in the titanium specimens indicated higher inflammation in the titanium specimens [12]. Due to the limited sample sizes in this study, and a short observation period, the difference of the extension and amount of inflammatory infiltrate between titanium and zirconia should be further examined. And other detection of immune-respond might be helpful in clarify the differences.

Statistical power calculation of the attachment percentage in this study equal 1. But to be able to conclude the clinical significant of inflammatory response, we suggested at least 20 samples per group should be included and we suggested longer observation time especially in gold alloy abutment to observe the clinical validity.

According to the result, clinical and surgical observations were in accordance with the attachment seen in the histological slide. In the case that very loose biopsy tissue was reported and the gap and displacement of tissue were described in histological slide, large area of inflammatory cells presented in both connective tissue and epithelium. On contrary, the case with no gingival inflammation was reported, harvesting tissue presented only some detachment, histological revealed some inflammatory cells presented.

Conclusion

In summary, the present study demonstrated that, with the experimental set up of 2 months healing period in a limited sample size, abutments made of titanium and zirconia promoted better attachment percentage whereas abutments made of gold alloy established significantly poorer attachment condition. Degree of inflammatory responses tended to be higher in gold alloy abutments compare to titanium abutments.

รายการอ้างอิง

1. Hermann, J.S., et al., *Biologic Width around one- and two-piece titanium implants.* Clin Oral Implants Res, 2001. **12**(6): p. 559-71.
2. Berglundh, T., et al., *The soft tissue barrier at implants and teeth.* Clin Oral Implants Res, 1991. **2**(2): p. 81-90.
3. Rompen, E., et al., *The effect of material characteristics, of surface topography and of implant components and connections on soft tissue integration: a literature review.* Clin Oral Implants Res, 2006. **17 Suppl 2**: p. 55-67.
4. Bidra, A.S. and P. Rungruanganunt, *Clinical outcomes of implant abutments in the anterior region: a systematic review.* J Esthet Restor Dent, 2013. **25**(3): p. 159-76.
5. Ekfeldt, A., B. Furst, and G.E. Carlsson, *Zirconia abutments for single-tooth implant restorations: a retrospective and clinical follow-up study.* Clin Oral Implants Res, 2011. **22**(11): p. 1308-14.
6. Scarano, A., et al., *Bacterial adhesion on commercially pure titanium and zirconium oxide disks: an in vivo human study.* J Periodontol, 2004. **75**(2): p. 292-6.
7. van Brakel, R., et al., *Early bacterial colonization and soft tissue health around zirconia and titanium abutments: an in vivo study in man.* Clin Oral Implants Res, 2011. **22**(6): p. 571-7.
8. Welander, M., I. Abrahamsson, and T. Berglundh, *The mucosal barrier at implant abutments of different materials.* Clin Oral Implants Res, 2008. **19**(7): p. 635-41.
9. Abrahamsson, I. and G. Cardaropoli, *Peri-implant hard and soft tissue integration to dental implants made of titanium and gold.* Clin Oral Implants Res, 2007. **18**(3): p. 269-74.
10. Abrahamsson, I., et al., *The mucosal attachment at different abutments. An experimental study in dogs.* J Clin Periodontol, 1998. **25**(9): p. 721-7.
11. Berglundh, T., et al., *Morphogenesis of the peri-implant mucosa: an experimental study in dogs.* Clin Oral Implants Res, 2007. **18**(1): p. 1-8.

12. Degidi, M., et al., *Inflammatory infiltrate, microvessel density, nitric oxide synthase expression, vascular endothelial growth factor expression, and proliferative activity in peri-implant soft tissues around titanium and zirconium oxide healing caps.* J Periodontol, 2006. **77**(1): p. 73-80.

13. van Brakel, R., et al., *Soft tissue response to zirconia and titanium implant abutments: an in vivo within-subject comparison.* J Clin Periodontol, 2012. **39**(10): p. 995-1001.

14. Vigolo, P., et al., *A 4-year prospective study to assess peri-implant hard and soft tissues adjacent to titanium versus gold-alloy abutments in cemented single implant crowns.* J Prosthodont, 2006. **15**(4): p. 250-6.

15. Linkevicius, T. and P. Apse, *Influence of abutment material on stability of peri-implant tissues: a systematic review.* Int J Oral Maxillofac Implants, 2008. **23**(3): p. 449-56.

16. Abrahamsson, I., et al., *The peri-implant hard and soft tissues at different implant systems. A comparative study in the dog.* Clin Oral Implants Res, 1996. **7**(3): p. 212-9.

17. Berglundh, T. and J. Lindhe, *Dimension of the periimplant mucosa. Biological width revisited.* J Clin Periodontol, 1996. **23**(10): p. 971-3.

18. Lindhe, J. and T. Berglundh, *The interface between the mucosa and the implant.* Periodontol 2000, 1998. **17**: p. 47-54.

19. Jansen, J.A., et al., *Ultrastructural study of epithelial cell attachment to implant materials.* J Dent Res, 1985. **64**(6): p. 891-6.

20. Jones, J.C., S.B. Hopkinson, and L.E. Goldfinger, *Structure and assembly of hemidesmosomes.* Bioessays, 1998. **20**(6): p. 488-94.

21. Listgarten, M.A., *Soft and hard tissue response to endosseous dental implants.* Anat Rec, 1996. **245**(2): p. 410-25.

22. Kohal, R.J., et al., *Loaded custom-made zirconia and titanium implants show similar osseointegration: an animal experiment.* J Periodontol, 2004. **75**(9): p. 1262-8.

23. Chai, W.L., et al., *A review of histomorphometric analysis techniques for assessing implant-soft tissue interface.* Biotech Histochem, 2011. **86**(4): p. 242-54.

24. Tomasi, C., et al., *Morphogenesis of peri-implant mucosa revisited: an experimental study in humans.* Clin Oral Implants Res, 2013.

25. Donath, K. and G. Breuner, *A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique.* J Oral Pathol, 1982. **11**(4): p. 318-26.

26. Chai, W.L., et al., *Contour analysis of an implant--soft tissue interface.* J Periodontal Res, 2013. **48**(5): p. 663-70.

27. Schwarz, F., et al., *Histological evaluation of different abutments in the posterior maxilla and mandible: an experimental study in humans.* J Clin Periodontol, 2013. **40**(8): p. 807-15.

28. Zitzmann, N.U., T. Berglundh, and J. Lindhe, *Inflammatory lesions in the gingiva following resective/non-resective periodontal therapy.* J Clin Periodontol, 2005. **32**(2): p. 139-46.

29. Pongnarisorn, N.J., et al., *Inflammation associated with implants with different surface types.* Clin Oral Implants Res, 2007. **18**(1): p. 114-25.

30. Berglundh, T., et al., *Histopathological observations of human periimplantitis lesions.* J Clin Periodontol, 2004. **31**(5): p. 341-7.

31. Loe, H. and J. Silness, *Periodontal Disease in Pregnancy. I. Prevalence and Severity.* Acta Odontol Scand, 1963. **21**: p. 533-51.

32. Canullo, L., et al., *Histological and Histomorphometrical Evaluation of Postextractive Sites Grafted with Mg-Enriched Nano-Hydroxyapatite: A Randomized Controlled Trial Comparing 4 Versus 12 Months of Healing.* Clin Implant Dent Relat Res, 2015.

33. Lang, N.P., T. Berglundh, and P. Working Group 4 of Seventh European Workshop on, *Periimplant diseases: where are we now?--Consensus of the Seventh European Workshop on Periodontology.* J Clin Periodontol, 2011. **38 Suppl 11**: p. 178-81.

34. Berglundh, T., A. Stavropoulos, and V.E.W.o.P. Working Group 1 of the, *Preclinical in vivo research in implant dentistry. Consensus of the eighth European workshop on periodontology.* J Clin Periodontol, 2012. **39 Suppl 12**: p. 1-5.

ภาคผนวก

Appendix1 Raw data; all samples

s	TL1	TL2	TLM	PMJE1	PMJE2	PMJEM	gap1	gap2	GAPM	Tgap1	Tgap2	TgapM	TATM
1	2418	2335.8	2376.9	388.5	370.7	379.6	585	571.4	578.2	0.288248	0.290774	0.289511	0.710489
1	2352	2337.68	2344.84	584.5	594.2	589.35	400	404	402	0.226308	0.23172	0.229014	0.770986
1	1344	1341.5	1342.75	315.2	392.5	353.85	0	0	0	0	0	0	1
1	1929	1925	1927	326.5	331.9	329.2	122	108.1	115.05	0.076131	0.067855	0.071993	0.928007
1	4578	4544.7	4561.35	502.2	482.8	492.5	692	693.9	692.95	0.169783	0.170831	0.170307	0.829693
1	2699	2693.9	2696.45	129.8	122.6	126.2	0	0	0	0	0	0	1
1	4657	4671.77	4664.385	415.2	435	425.1	1333	1309.77	1321.385	0.314253	0.309144	0.311698	0.688302
1	3995	3972.9	3983.95	50	40	45	766	770.1	768.05	0.19417	0.19581	0.19499	0.80501
1	3286	3249.4	3267.7	750.5	741.4	745.95	1118	1103	1110.5	0.440939	0.439793	0.440366	0.559634
1	2390	2414.5	2402.25	116.8	131.8	124.3	404	418.7	411.35	0.177723	0.183423	0.180573	0.819427
1	3111	3072.3	3091.65	623.2	608.8	616	146	131.4	138.7	0.058686	0.053339	0.056013	0.943987
1	2303	2293.32	2298.16	82.75	87.62	85.185	384	384.7	384.35	0.172953	0.174412	0.173683	0.826317
1	3198	3179.91	3188.955	187.9	201.7	194.8	86	66.21	76.105	0.02857	0.022231	0.025401	0.974599
1	3662	3663	3662.5	528	526.5	527.25	379	388.5	383.75	0.120932	0.123864	0.122398	0.877602
1	4449	4387	4418	425.5	365.2	395.35	3081.5	3058.5	3070	0.765875	0.76048	0.763178	0.236822
1	2683	2694.3	2688.65	204.5	211.3	207.9	97	112	104.5	0.039137	0.045107	0.042122	0.957878
1	~~3178~~	~~3174.1~~	~~3176.05~~	~~327~~	~~331.9~~	~~329.45~~	~~2173~~	~~2162.6~~	~~2167.8~~	~~0.762189~~	~~0.760889~~	~~0.761539~~	~~0.238461~~
1	~~2544~~	~~2546.57~~	~~2545.285~~	~~112.4~~	~~112.9~~	~~112.65~~	~~2402.6~~	~~2414.2~~	~~2408.4~~	~~0.988074~~	~~0.992~~	~~0.990037~~	~~0.009963~~
1	~~2859~~	~~2430~~	~~2644.5~~	~~0~~	~~0~~	~~0~~	~~430~~	~~414.4~~	~~422.2~~	~~0.150402~~	~~0.170535~~	~~0.160469~~	~~0.839531~~
1	~~2859~~	~~2960~~	~~2909.5~~	~~160.6~~	~~146~~	~~153.3~~	~~185~~	~~194.8~~	~~189.9~~	~~0.068559~~	~~0.069225~~	~~0.068892~~	~~0.931108~~
2	3556	3509.9	3532.95	737.7	721.8	729.75	195	175.8	185.4	0.069191	0.063054	0.066122	0.933878
2	3137	3116.4	3126.7	373.8	359.4	366.6	0	0	0	0	0	0	1
2	3101	3083.5	3092.25	593.9	579.3	586.6	1923	1920	1921.5	0.767022	0.766712	0.766867	0.233133
2	3640	3596.5	3618.25	300	280	290	2567	2574.3	2570.65	0.768563	0.77621	0.772386	0.227614
2	2624	2614.9	2619.45	186.6	187.9	187.25	0	0	0	0	0	0	1
2	2731	2726.2	2728.6	0	0	0	282	287.2	284.6	0.103259	0.105348	0.104303	0.895697
2	3772	3750.4	3761.2	271.2	269.5	270.35	1514	1508.6	1511.3	0.432473	0.433394	0.432933	0.567067
2	2763	2777.4	2770.2	275.1	271.5	273.3	285	296.9	290.95	0.114554	0.11848	0.116517	0.883483
2	3207	3207.96	3207.48	595.9	614.2	605.05	44.7	44.88	44.79	0.017119	0.017303	0.017211	0.982789
2	3754	3721.5	3737.75	152.2	146.8	149.5	726	694.7	710.35	0.201566	0.194338	0.197952	0.802048
2	3255	3249.6	3252.3	567.2	571.6	569.4	0	0	0	0	0	0	1
2	3447	3408.7	3427.85	618.3	608.5	613.4	1207.7	1179.2	1193.45	0.426945	0.421113	0.424029	0.575971
2	2254	2250.96	2252.48	63.47	48.92	56.195	68	78.04	73.02	0.031043	0.03544	0.033241	0.966759
2	3333	3294.4	3313.7	331.8	341.2	336.5	452	457.2	454.6	0.150606	0.154815	0.152711	0.847289
2	2922	2915.4	2918.7	294	297.9	295.95	142	138.5	140.25	0.054033	0.052913	0.053473	0.946527
2	3074	3046.21	3060.105	295.6	301.7	298.65	223	208.71	215.855	0.080262	0.076046	0.078154	0.921846
2	3359	3331.7	3345.35	973.1	969	971.05	305	304.7	304.85	0.127834	0.128963	0.128398	0.871602
2	3030	3015.3	3022.65	200.1	190.4	195.25	585	589.2	587.1	0.206721	0.208574	0.207647	0.792353
2	2076	2064.5	2070.25	468.3	466.4	467.35	157	155.5	156.25	0.097655	0.097303	0.097479	0.902521
2	4212	4186.9	4199.45	876.7	885.9	881.3	1086	1076	1081	0.325608	0.325962	0.325785	0.674215

3	3696	3688.6	3692.3	172	166.1	169.05	3318	3301.5	3309.75	0.941544	0.93726	0.939402	0.060598
3	1861	1836.8	1848.9	0	0	0	1617	1341	1479	0.868888	0.730074	0.799481	0.200519
3	3178	3191	3184.5	753.2	758	755.6	0	0	0	0	0	0	1
3	1124	1123.8	1123.9	87	87	87	735.2	735	735.1	0.708968	0.708912	0.70894	0.29106
3	3855	3820.1	3837.55	526.5	534.9	530.7	452	418.2	435.1	0.135797	0.127298	0.131548	0.868452
3	4721	4744.4	4732.7	793.5	793.6	793.55	1933	1924.8	1928.9	0.492171	0.487192	0.489682	0.510318
3	2705	2647.63	2676.315	814.1	808.8	811.45	424	390.43	407.215	0.224232	0.212325	0.218279	0.781721
3	2750	2740.9	2745.45	0	0	0	1324	1328.9	1326.45	0.481455	0.484841	0.483148	0.516852
3	1711	1726.1	1718.55	0	0	0	590	594.1	592.05	0.344828	0.344186	0.344507	0.655493
3	3255	3266.1	3260.55	214.7	210.7	212.7	424	429.4	426.7	0.13946	0.140538	0.139999	0.860001
3	2133	2176.6	2154.8	793.4	789.7	791.55	161	175.9	168.45	0.120185	0.12683	0.123507	0.876493
3	2021	2025.9	2023.45	165.6	165.8	165.7	628	623.1	625.55	0.338471	0.334982	0.336727	0.663273
3	3335	3296.2	3315.6	243.4	244	243.7	1536.6	1544.4	1540.5	0.497024	0.505996	0.50151	0.49849
3	2483	2475	2479	391.9	392.4	392.15	910.7	897.3	904	0.435512	0.430856	0.433184	0.566816
3	3247	2905	3076	338.7	222.5	280.6	1351	1370.3	1360.65	0.464533	0.510829	0.487681	0.512319
3	2480	2435.6	2457.8	567.7	543	555.35	1550.3	1550.2	1550.25	0.810699	0.819085	0.814892	0.185108
3	2392.3	2371	2381.65	124.1	111	117.55	229	237.4	233.2	0.100961	0.105044	0.103003	0.896997
3	3284	3228.44	3256.22	0	0	0	1199.1	1165.74	1182.42	0.365134	0.361085	0.363109	0.636891
3	~~2629~~	~~2667.4~~	~~2648.2~~	~~187.5~~	~~176.3~~	~~181.9~~	~~1781.2~~	~~1770.9~~	~~1776.05~~	~~0.729552~~	~~0.710891~~	~~0.720221~~	~~0.279779~~
3	~~3182~~	~~3186.1~~	~~3184.05~~	~~211.3~~	~~205.9~~	~~208.6~~	~~2798.6~~	~~2799~~	~~2798.8~~	~~0.942068~~	~~0.939199~~	~~0.940633~~	~~0.059367~~

Raw data; all samples

abutment type	sample	slide of sample	GI	Surgical score	TATM	infE	infC
1	1	1a	1	2	0.710489	1	2
1	1	1b	1	2	0.770986	1	1
1	1	1c	1	2	1	1	1
1	1	1d	1	2	0.928007	1	1
1	2	2a	0	1	0.829693	2	2
1	2	2b	0	1	1	2	2
1	2	2c	0	1	0.688302	2	2
1	2	2d	0	1	0.80501	3	2
1	3	3a	0	2	0.559634	3	2
1	3	3b	0	2	0.819427	2	2
1	3	3c	0	2	0.943987	2	2
1	3	3d	0	2	0.826317	2	2
1	4	4a	1	3	0.974599	1	1
1	4	4b	1	3	0.877602	1	1
1	4	4c	1	3	0.236822	2	1
1	4	4d	1	3	0.957878	2	1
1	5	5a	1	2	#NULL!	#NULL!	#NULL!
1	5	5b	1	2	#NULL!	#NULL!	#NULL!
1	5	5c	1	2	#NULL!	#NULL!	#NULL!
1	5	5d	1	2	#NULL!	#NULL!	#NULL!

2	6	6a	1	2	0.933878	1	1
2	6	6b	1	2	1	1	1
2	6	6c	1	2	0.233133	1	2
2	6	6d	1	2	0.227614	1	2
2	7	7a	0	2	1	2	2
2	7	7b	0	2	0.895697	2	2
2	7	7c	0	2	0.567067	2	2
2	7	7d	0	2	0.883483	2	2
2	8	8a	1	2	0.982789	2	2
2	8	8b	0	2	0.802048	3	3
2	8	8c	1	2	1	2	3
2	8	8d	0	2	0.575971	3	2
2	9	9a	0	1	0.966759	2	2
2	9	9b	1	1	0.847289	2	2
2	9	9c	0	1	0.946527	1	1
2	9	9d	1	1	0.921846	1	2
2	10	10a	0	2	0.871602	1	2
2	10	10b	0	2	0.792353	2	2
2	10	10c	0	2	0.902521	1	2
2	10	10d	0	2	0.674215	2	2
3	11	11a	1	3	0.060598	3	3
3	11	11b	1	3	0.200519	3	3
3	11	11c	1	3	1	3	3
3	11	11d	1	3	0.29106	2	3
3	12	12a	1	2	0.868452	3	3
3	12	12b	1	2	0.510318	3	3
3	12	12c	1	2	0.78	1	1
3	12	12d	1	2	0.516852	2	2
3	13	13a	1	2	0.655493	2	2
3	13	13b	1	2	0.860001	2	1
3	13	13c	1	2	0.876493	2	2
3	13	13d	1	2	0.663273	2	2
3	14	14a	1	2	0.49849	2	2
3	14	14b	1	2	0.566816	2	2
3	14	14c	1	2	0.512319	1	1
3	14	14d	1	2	0.185108	1	1
3	15	15a	1	3	0.896997	2	2
3	15	15b	1	3	0.636891	2	2
3	15	15c	1	3	0.279779	#NULL!	#NULL!
3	15	15d	1	3	0.059367	#NULL!	#NULL!

Raw data; Case which patient received two type of abutment

case	abutment type	sample	slide of sample	GI	Surgical score	TATM	INFE	INFC
a	1	1	1a	1	2	0.710489	1	2
a	1	1	1b	1	2	0.770986	1	1
a	1	1	1c	1	2	1	1	1
a	1	1	1d	1	2	0.928007	1	1
a	2	6	6a	1	2	0.933878	1	1
a	2	6	6b	1	2	1	1	1
a	2	6	6c	1	2	0.233133	1	2
a	2	6	6d	1	2	0.227614	1	2
b	1	2	2a	0	1	0.829693	2	2
b	1	2	2b	0	1	1	2	2
b	1	2	2c	0	1	0.688302	2	2
b	1	2	2d	0	1	0.80501	3	2
b	2	7	7a	0	2	1	2	2
b	2	7	7b	0	2	0.895697	2	2
b	2	7	7c	0	2	0.567067	2	2
b	2	7	7d	0	2	0.883483	2	2
c	2	8	8a	1	2	0.982789	2	2
c	2	8	8b	0	2	0.802048	3	3
c	2	8	8c	1	2	1	2	3
c	2	8	8d	0	2	0.575971	3	2
c	3	12	12a	1	2	0.868452	3	3
c	3	12	12b	1	2	0.510318	3	3
c	3	12	12c	1	2	0.78	1	1
c	3	12	12d	1	2	0.516852	2	2
d	1	4	4a	1	3	0.974599	1	1
d	1	4	4b	1	3	0.877602	1	1
d	1	4	4c	1	3	0.236822	2	1
d	1	4	4d	1	3	0.957878	2	1
d	3	15	15a	1	3	0.896997	2	2
d	3	15	15b	1	3	0.636891	2	2
d	3	15	15c	1	3	0.279779	#NULL!	#NULL!
d	3	15	15d	1	3	0.059367	#NULL!	#NULL!
e	1	5	5a	1	2	#NULL!	#NULL!	#NULL!
e	1	5	5b	1	2	#NULL!	#NULL!	#NULL!
e	1	5	5c	1	2	#NULL!	#NULL!	#NULL!
e	1	5	5d	1	2	#NULL!	#NULL!	#NULL!
e	3	14	14a	1	2	0.49849	2	2
e	3	14	14b	1	2	0.566816	2	2
e	3	14	14c	1	2	0.512319	1	1
e	3	14	14d	1	2	0.185108	1	1

Appendix2 Inferential statistics

GI Variable

Ranks

	material	N	Mean Rank
maxGI	titanium	5	7.00
	zirconia	5	7.00
	gold	5	10.00
	Total	15	

Test Statistics[a,b]

	maxGI
Chi-Square	2.545
df	2
Asymp. Sig.	.280

a. Kruskal Wallis Test
b. Grouping Variable: material

maxGI

	Observed N	Expected N	Residual
0	4	7.5	-3.5
1	11	7.5	3.5
Total	15		

Test Statistics

	maxGI
Chi-Square	3.267[a]
df	1
Asymp. Sig.	.071

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 7.5.

Surgical Score Variable

Ranks

	material	N	Mean Rank
surgscale	titanium	5	7.60
	zirconia	5	6.30
	gold	5	10.10
	Total	15	

Test Statistics[a,b]

	surgscale
Chi-Square	2.678
df	2
Asymp. Sig.	.262

a. Kruskal Wallis Test
b. Grouping Variable: material

Appendix3 Analytical statistic

Attachment percentage variable

```
> anova(null.lmm,my.lmm) #overall test of the effect
refitting model(s) with ML (instead of REML)
Data: data
Models:
null.lmm: TATM ~ 1 + (1 | sample)
my.lmm: TATM ~ abutment.fac + (1 | sample)
         Df     AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
null.lmm  3 16.0428 22.119 -5.0214  10.0428
my.lmm    5  8.7813 18.908  0.6093  -1.2187 11.261      2   0.003586 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> summary(my.lmm) # summarized the output
Linear mixed model fit by REML ['lmerMod']
Formula: TATM ~ abutment.fac + (1 | sample)
   Data: data

REML criterion at convergence: 10.5

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.3256 -0.4211  0.2337  0.6258  1.8541

Random effects:
 Groups   Name        Variance  Std.Dev.
 sample   (Intercept) 0.0001559 0.01249
 Residual             0.0604028 0.24577
Number of obs: 56, groups:  sample, 14

Fixed effects:
              Estimate Std. Error t value
(Intercept)   0.808047   0.061759  13.084
abutment.fac2 -0.006808   0.082858  -0.082
abutment.fac3 -0.262106   0.082858  -3.163

Correlation of Fixed Effects:
            (Intr) abtm.2
abutmnt.fc2 -0.745
abutmnt.fc3 -0.745  0.556
```

```
> confint(my.lmm,method="Wald")
                   2.5 %      97.5 %
.sig01                NA          NA
.sigma                NA          NA
(Intercept)    0.6870020  0.92909217
abutment.fac2 -0.1692066  0.15559136
abutment.fac3 -0.4245048 -0.09970675
```

Inflammatory extent grade

```
> anova(null.ord.lmm, grade.ord.lmm)
Likelihood ratio tests of cumulative link models:

              formula:                                  link: threshold:
null.ord.lmm  grade.fac ~ 1 + (1 | sample)              logit flexible
grade.ord.lmm grade.fac ~ abutment.fac + (1 | sample) logit flexible

              no.par    AIC  logLik LR.stat df Pr(>Chisq)
null.ord.lmm       3 101.75 -47.877
grade.ord.lmm      5 103.74 -46.868  2.0172  2     0.3647
> |
> summary(grade.ord.lmm)
Cumulative Link Mixed Model fitted with the Laplace approximation

formula: grade.fac ~ abutment.fac + (1 | sample)
data:    data

 link  threshold nobs logLik AIC    niter    max.grad cond.H
 logit flexible  54   -46.87 103.74 145(565) 5.83e-06 7.3e+01

Random effects:
 Groups Name        Variance Std.Dev.
 sample (Intercept) 3.195    1.787
Number of groups:  sample 14

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
abutment.fac2  -0.1341     1.4246  -0.094    0.925
abutment.fac3   1.6455     1.4535   1.132    0.258

Threshold coefficients:
    Estimate Std. Error z value
1|2  -0.7489     1.0913  -0.686
2|3   3.0970     1.2240   2.530
(6 observations deleted due to missingness)
> |
> OR.INFE
         effect coefficients      95%LL      95%UP
1           1|2    0.4728924 0.05569277   4.015373
2           2|3   22.1313950 2.00954427 243.736180
3 abutment.fac2    0.8745125 0.05359550  14.269333
4 abutment.fac3    5.1834009 0.30016619  89.509231
> |
```

Inflammatory cellularity grade

```
> anova(null.ord.lmm, count.ord.lmm)
Likelihood ratio tests of cumulative link models:

               formula:                                    link: threshold:
null.ord.lmm   count.fac ~ 1 + (1 | sample)                logit flexible
count.ord.lmm  count.fac ~ abutment.fac + (1 | sample)     logit flexible

              no.par    AIC   logLik LR.stat df Pr(>Chisq)
null.ord.lmm       3 92.481 -43.241
count.ord.lmm      5 93.300 -41.650  3.1814  2     0.2038
> 
> summary(count.ord.lmm)
Cumulative Link Mixed Model fitted with the Laplace approximation

formula: count.fac ~ abutment.fac + (1 | sample)
data:    data

 link  threshold nobs logLik AIC   niter    max.grad cond.H
 logit flexible  54   -41.65 93.30 144(705) 2.38e-07 9.9e+01

Random effects:
 Groups Name        Variance Std.Dev.
 sample (Intercept) 3.924    1.981
Number of groups:  sample 14

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
abutment.fac2    2.028      1.595   1.272   0.2034
abutment.fac3    2.883      1.658   1.738   0.0821 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Threshold coefficients:
    Estimate Std. Error z value
1|2  -0.1427     1.1918  -0.120
2|3   4.6749     1.4845   3.149
(6 observations deleted due to missingness)
> 
> OR.INFC
         effect coefficients      95%LL       95%UP
1           1|2    0.8670416 0.08386573    8.963865
2           2|3  107.2177837 5.84253253 1967.580512
3 abutment.fac2    7.5983694 0.33373906  172.995085
4 abutment.fac3   17.8598526 0.69260050  460.545921
> 
```

Appendix4 Consent form

เอกสารยินยอมเข้าร่วมการวิจัย (Consent Form)

การวิจัยเรื่อง การศึกษาลักษณะทางจุลกายวิภาคศาสตร์ และ ปฏิกิริยาการอักเสบของเนื้อเยื่อรอบหลักยึดที่ทำจากวัสดุต่างชนิดในมนุษย์

ข้าพเจ้า (นาย, นาง, นางสาว, เด็กชาย, เด็กหญิง)...

อยู่บ้านเลขที่..........................ถนน..ตำบล/แขวง...

อำเภอ/เขต..จังหวัด...รหัสไปรษณีย์..............

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้

1. ข้าพเจ้าได้รับทราบรายละเอียดข้อมูลคำอธิบายสำหรับอาสาสมัครที่เข้าร่วมในการวิจัย รวมทั้งได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการทำวิจัย อันตรายหรืออาการที่อาจเกิดขึ้นจากการทำวิจัยหรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียดและมีความเข้าใจดีแล้ว
2. ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ
3. ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับและจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆ ที่เกี่ยวข้องกระทำได้เฉพาะกรณีจำเป็นด้วยเหตุผลทางวิชาการเท่านั้น และผู้วิจัยรับรองว่าหากเกิดอันตรายใดๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่คิดมูลค่า
4. ข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าร่วมในโครงการวิจัยนี้เมื่อใดก็ได้และการบอกเลิกการเข้าร่วมการวิจัยนี้จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะพึงได้รับต่อไป

ข้าพเจ้าจึงสมัครใจเข้าร่วมโครงการวิจัยนี้ตามที่ระบุในเอกสารข้อมูลคำอธิบายสำหรับอาสาสมัคร และได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ และได้รับสำเนาเอกสารใบยินยอมที่ข้าพเจ้าลงนามและลงวันที่ และเอกสารยกเลิกการเข้าร่วมวิจัย อย่างละ 1 ฉบับ เป็นที่เรียบร้อยแล้ว

ลงนาม.. ผู้ยินยอม

(...)

วันที่................เดือน...............................พ.ศ.............................

ลงนาม.. พยาน

(..)

วันที่................................เดือน...............................พ.ศ............................

ลงนาม... ผู้วิจัยหลัก

(...)

วันที่................................เดือน...............................พ.ศ..............................

.

ข้าพเจ้าไม่สามารถอ่านหนังสือได้

แต่ผู้วิจัยได้อ่านข้อความในใบยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจดีแล้ว ข้าพเจ้าจึงลงนาม
หรือประทับลายนิ้วหัวแม่มือขวาของข้าพเจ้าในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม...ผู้ยินยอม

(..)

วันที่................................เดือน...............................พ.ศ..............................

ลงนาม...พยาน

(...)

วันที่................................เดือน...............................พ.ศ..............................

ลงนาม...ผู้วิจัยหลัก

(...)

วันที่................................เดือน.................................พ.ศ..............................

ในกรณีที่ผู้ถูกทดลองยังไม่บรรลุนิติภาวะ จะต้องได้รับการยินยอมจากผู้ปกครองหรือผู้อุปการะ
โดยชอบด้วยกฎหมาย

ลงนาม.. ผู้ปกครอง

(..)

วันที่................................เดือน...............................พ.ศ..............................

ลงนาม.. พยาน

(..)

วันที่................................เดือน...............................พ.ศ...............................

ลงนาม.. ผู้วิจัยหลัก

(..)

วันที่................................เดือน...............................พ.ศ............................

ประวัติผู้เขียนวิทยานิพนธ์

Name: Teeratida Sampatanukul

Date of birth: 2 November 1988

Institution Attended:

2006-2012 Doctor of Dental Surgery, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

2014 Preceptorship Program in Advanced Implantology, School of Dentistry, University of California Los Angeles, CA, USA