การศึกษาแบคทีเรียโดยรอบรากเทียมที่มีการออกแบบส่วนผ่านเนื้อเยื่ออ่อนแตกต่างกันสามแบบ

นางสาวรินทร์ลภัส ศรีปิติโรจน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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The Microbiota Associating with Three Different Transmucosal Implant Designs

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A Thesis Submitted in Partial Fulfillment of the Requirements

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Thesis Title	The Microbiota Associating with Three Different Transmucosal Implant Designs
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รินทร์ลภัส ศรีปิติโรจน์ : การศึกษาแบคทีเรียโดยรอบรากเทียมที่มีการออกแบบส่วนผ่าน เนื้อเยื่ออ่อนแตกต่างกันสามแบบ (The Microbiota Associating with Three Different Transmucosal Implant Designs) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : อ. ทญ.ดร. อัญชลี วัชรักษะ, 50 หน้า.

วัตถุประสงค์ เพื่อศึกษาแบคทีเรียโดยรอบรากเทียม คือ พอร์ไฟโลโมนัส จินจิวาลิส (*Porphyromonas gingivalis, Pg*) ทรีโพนีมา เด็นติโคลา (*Treponema denticola, Td*) และ แทนเนอเรลลา ฟอร์ไซเทีย (*Treponema forsythia, Tf*)ที่มีการออกแบบส่วนผ่านเนื้อเยื่ออ่อนที่ แตกต่างกัน **วิธีการทดลอง** แบ่งคนไข้ 45 คน ออกเป็น 3 กลุ่มตามชนิดของส่วนผ่านเนื้อเยื่ออ่อน คือ กลุ่มที่มีเส้นผ่านศูนย์กลางของส่วนผ่านเนื้อเยื่ออ่อนเท่ากับเส้นผ่านศูนย์กลางของรากเทียม จำนวน 15 คน กลุ่มที่มีเส้นผ่านศูนย์กลางของส่วนผ่านเนื้อเยื่ออ่อนสั้นกว่าเส้นผ่านศูนย์กลางของ รากเทียม จำนวน 15 คน และกลุ่มที่มีส่วนผ่านเนื้อเยื่ออ่อนอยู่เหนือระดับกระดูก จำนวน 15 คน แล้วใช้กระดาษปลายแหลมซับน้ำจากร่องเหงือกบริเวณรากเทียม จากนั้นนำมาสกัดดีเอ็นเอ แล้ว นำดีเอ็นเอที่สกัดได้มาทดสอบโดยวิธีปฏิกิริยาลูกโซ่พอลิเมอเรส โดยใช้ไพรเมอร์ที่มีความจำเพาะ ต่อแบคทีเรียที่ก่อโรคปริทันต์ คือ พอร์ไฟโลโมนัส จินจิวาลิส ทรีโพนีมา เด็นติโคลา และ แทนเนอ เรลลา ฟอร์ไซทัส ในการทดสอบเพื่อหาชนิดของแบคทีเรียโดยรอบรากเทียมทั้ง 3 กลุ่ม ผลการ ทดลองที่ได้วิเคราะห์ด้วยสถิติไคสแควร์ที่ระดับนัยสำคัญ 0.05 **ผลการทดลอง** ไม่มีความแตกต่าง อย่างมีนัยสำคัญทางสถิติของสัดส่วนของแบคทีเรียทั้ง 3 ชนิด ระหว่างกลุ่มของส่วนผ่านเนื้อเยื่อ อ่อนชนิดต่างๆกัน แต่กลุ่มที่มีเส้นผ่านศูนย์กลางของส่วนผ่านเนื้อเยื่ออ่อนเท่ากับเส้นผ่าน ศูนย์กลางของรากเทียมและกลุ่มที่มีเส้นผ่านศูนย์กลางของส่วนผ่านเนื้อเยื่ออ่อนสั้นกว่าเส้นผ่าน ศูนย์กลางของรากเทียมพบความแตกต่างอย่างมีนัยสำคัญทางสถิติของแบคทีเรียทั้ง 3 ชนิด **สรุป** แบคทีเรียที่ก่อให้เกิดโรคปริทันต์เป็นส่วนหนึ่งของแบคทีเรียที่พบได้ตามปกติในช่องปาก และ สามารถพบได้ในบริเวณโดยรอบรากเทียมที่อยู่ในภาวะปกติ

สาขาวิชา ทันตกรรมบูรณะเพื่อความสวยงาม ลายมือชื่อนิสิต.. ....

และทันตกรรมรากเทียม ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.............

ปีการศึกษา 2555

5276157032 : MAJOR Esthetic Restorative and Implant Dentistry

KEYWORDS : microgap, implant-abutment connection, peri-implantitis, red complex bacteria, microbiota

RINRAPAT SRIPITIROJ : THE MICROBIOTA ASSOCIATING WITH THREE DIFFERENT TRANSMUCOSAL IMPLANT DESIGN. ADVISOR : ANJALEE VACHARAKSA, Ph.D., 50 pp.

Objective To investigate the putative pathogenic bacteria, *Porphyromonas gingivalis (Pg), Treponema denticola (Td), or Tanerella forsythia (Tf)*, associating with implant-supported dental prostheses using differential transmucosal designs. **Methodology** Forty-five partially edentulous patients who received implant-supported fixed partial prostheses were randomly selected and categorized into three groups based on the transmucosal implant-abutment connections, including the platform-matching abutments (n = 15), platform-switching abutments (n = 15), and tissue-level dental implants (n = 15). To obtain subgingival bacterial samples, four paper points were inserted under light pressure in healthy peri-implant sulci, and DNA was extracted from the samples. *Pg-, Td-, or Tf* -specific DNA was detected by endpoint PCR. **Results** There was no statistically significant differences (P > 0.05) in the frequencies of the *Pg, Tf,* or *Td* associating with differential transmucosal designs under healthy conditions. However, the proportion of *Tf* associating with platform-matching abutments or platform-switching abutments was substantially higher than *Pg* or *Td*. The chi-square test showed significant differences (P < 0.05) in the frequencies of these bacteria in the groups restored with platform-matching abutments or platform-switching abutments, but not tissue-level dental implants. **Conclusion** periodontal pathogens are part of the normal resident microbiota and may be found at healthy peri-implant sites.

Field of Study: <u>Esthetic Restorative and</u>   Student's Signature...................................

                     <u>Implant Dentistry</u>   Advisor's Signature…………………………

Academic Year: <u>2012</u>

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CHAPTER I

INTRODUCTION

Rationale and Significance of the problem

Implant-supported prostheses have been widely used for teeth substitution to restore functions and esthetics. Implant surface modification can primarily improve the osseointegration and its initial stability (Heuer, Stiesch et al. 2011). However, after the first year in function, dental implants restored with conventional, or platform-matching, transmucosal prostheses may result in crestal bone loss for up to 2 mm (Cardaropoli, Lekholm et al. 2006). Different transmucosal designs might affect functional bone remodeling. Clinical and radiographic examination showed that platform-switching abutment design resulted in more favorable peri-implant tissue response including tight connective tissue arrangement and less crestal bone loss when compared to the platform-matching design. That may be because the platform-switching design shifted the stress concentration area from the alveolar bone crest and bone-implant interface (Maeda, Miura et al. 2007). There are many factors relating to peri-implant bone remodeling. Yet, it is not clear whether the functional force was the only cause of crestal bone loss. In fact, bacterial infection might also play a role in bone remodeling process. The peri-implant ecology at the implant-tissue interface apparently influenced oral bacteria colonizing the implant-supported prostheses (Canullo, Quaranta et al. 2010).

Therefore, the purpose of this study was to investigate whether differential transmucosal designs could be associated to the specific peri-implant microbiota.

Research Question

Was specific oral microbiota associated with dental implant restored with differential transmucosal abutments connection?

Objective

To investigate the specific oral microbiota associating dental implants restored with the platform-switching prosthesis connection as compared to dental implants restored with platform-matching prostheses, and tissue-level dental implants.

Research Hypothesis

Null hypothesis:

There is no significant difference in the presence of *Porphyromonas gingivalis (Pg), Treponema denticola (Td), and Tannerella forsythia (Tf)* associating with three transmucosal abutment designs.

Alternative hypothesis:

There is a significant difference in the presence of *Porphyromonas gingivalis (Pg), Treponema denticola (Td), and Tannerella forsythia (Tf)* associating with three

transmucosal abutment designs.

Research design

Experimental research

CHAPTER II

LITERATURE REVIEW

Successful dental implants required osseointegrated implant with healthy soft tissue. The mucosal tissue of osseointegrated dental implants and periodontal tissue have similar functional structures including the junctional epithelium, which the epithelial cells formed hemidesmosomes to both tooth and titanium surface (Gould, Westbury et al. 1984), and the connective tissue compartment (Klinge, Hultin et al. 2005). However, the connective tissue that established the mucosal seal of the dental implants composed of dense-circular connective tissue fibers with less vascularization, fewer scattered fibroblasts, and greater amounts of collagen than the gingiva around teeth (Berglundh, Lindhe et al. 1994; Rompen, Domken et al. 2006). This tissue barrier called "biological width" is a protecting zone of the supporting bone from the bacterial penetration (Klinge, Hultin et al. 2005; Rompen, Domken et al. 2006).

The different transmucosal designs result in different location of the microgap. Historically, matching-platform abutment was attached at the outer edge of the bone level implant to restore the tooth (Fig.1A). The implant restored with matching-platform abutment therefore have implant-transmucosal abutment interface located close to the level of the crestal bone (O'Mahony, MacNeill et al. 2000). Component connection at the bone level could affect the crestal bone resorption and recession of the epithelium due to

the microgap between the prosthetic component and the dental implant (O'Mahony, MacNeill et al. 2000; Oh, Yoon et al. 2002). When wider-diameter implants were connected with smaller-diameter abutment, however, the platform-switched design has demonstrated a favorable change in the crestal bone height around the bone level dental implants (Fig.1B). Several studies have reported that the platform-switching design could improve crestal bone preservation (Canullo, Pace et al. 2011).
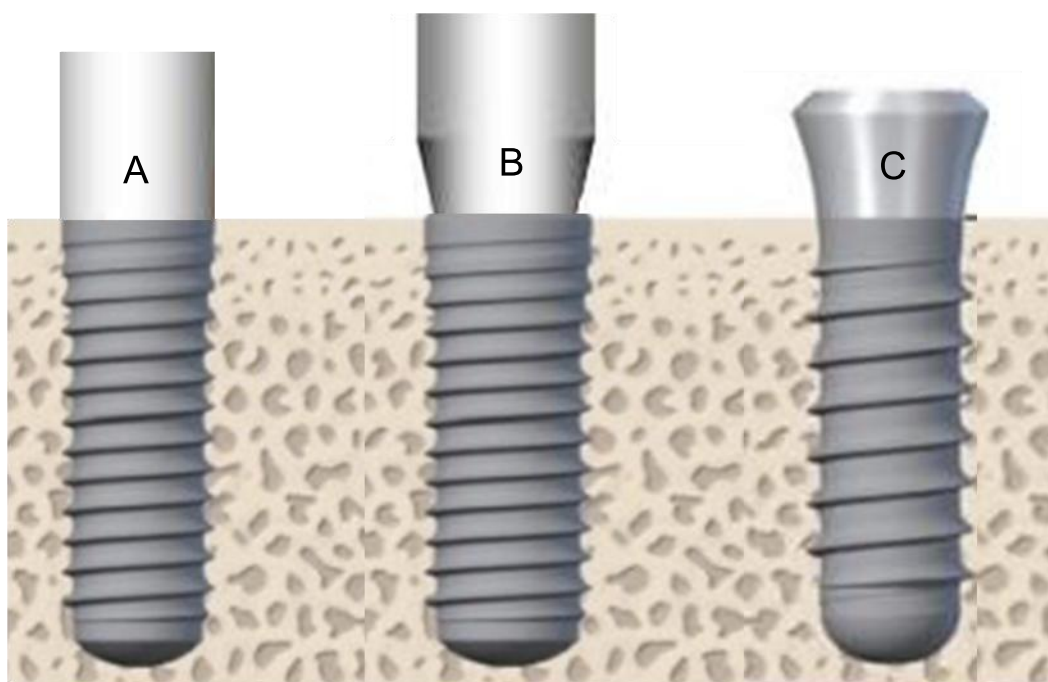
Figure 1. Transmucosal Implant-abutment Connection

A. Platform-matching abutment design, B. Platform-switching abutment design,

C. Tissue-level dental implant

Thus, the platform-switching design repositioned the outer edge of the implant-abutment interface inwardly and might prevent loss of peri-implant tissue (Canullo, Quaranta et al. 2010) since stress transferred onto crestal bone was decreased. Moreover, These

results also related to horizontally biologic width reestablishment (Canullo, Pace et al. 2011). The one-piece, nonsubmerged design of the dental implants (Fig.1C) placed the prosthetic component connection above the alveolar crest and therefore this design have a continuous surface at the crestal bone area that might provide additional benefits. Collectively, the designs of transmucosal abutments selectively position the prosthetic connection in relative to peri-implant tissue, and therefore affect tissue responses.

The presence or absence of a microgap was the important factor which associated with the bacterial colonization that influences hard and soft tissue dimension around dental implant (Hermann, Buser et al. 2001). Microgap between the component connections might predict the type of oral biofilms on the dental implants. Similar to any surfaces in the oral cavity, the transmucosal abutment surfaces were susceptible for bacterial colonization of microbial biofilms (Klinge, Hultin et al. 2005). The biofilm of dental implant materials and designs demonstrated distinct bacterial compositions suggesting the selectivity of the bacterial adhesion process (Mombelli, Buser et al. 1988; van Winkelhoff, Goene et al. 2000). The initial bacterial attachment process involved specific interaction between complementary surface components (Teughels, Van Assche et al. 2006), also the characteristics of the interacting surfaces, which is hydrophobicity, of a particular bacteria (Satou, Fukunaga et al. 1988), and the surface free-energy (Quirynen, Marechal et al. 1989; Teughels, Van Assche et al. 2006). Thus,

both salivary proteins and implant surface structures affected the early colonization pattern of bacteria on dental implants (Mombelli, Buser et al. 1988; van Winkelhoff, Goene et al. 2000). Salivary pellicle attached on titanium or hydroxyapatite surfaces including enamel differently upon ion distribution and charges of these surfaces. The surface of titanium was coated with an oxide layer that resulted in decrease affinity for charged salivary proteins (Wolinsky, de Camargo et al. 1989), therefore the surface free energy of titanium implants facilitates the formation of bacterial biofilms (Teughels, Van Assche et al. 2006). In addition, the total number of bacteria on rough surfaces was higher than smooth surfaces because of the increase of surface area and the wettability of the rough surfaces (Drake, Paul et al. 1999).

The oral biofilm of the dental implants are likely to be influenced by the ecosystem previously established in the oral cavity. For example, the developing microbiota around newly placed dental implants resembled the microflora of naturally remaining teeth in partially edentulous subjects (Leonhardt, Adolfsson et al. 1993; Mombelli, Marxer et al. 1995). A cross-sectional study demonstrated the increase complexity of the microbiota on implants with longer loading time or history of periodontal or peri-implant infection (Lee, Maiden et al. 1999). Implant-associated microbiota appeared immediately post dental implant installation and different microorganisms were presented at the sites (van Winkelhoff, Goene et al. 2000) and in the fluid around teeth and implants in less than an hour later (Furst, Salvi et al. 2007).

The submucosal microbiota around implant sites at 1 month were dominated with *P. micros, F. nucleatum*, and *P. intermedia* (van Winkelhoff, Goene et al. 2000).

Differential bacterial biofilms could be observed throughout the process of prosthetic connection on dental implants. The microbiota of oral implants in edentulous patients apparently matured within one week post placement and maintained through the 6 months period (Mombelli, van Oosten et al. 1987). In partially edentulous individuals, within 30 minutes after transmucosal implant placement, microbial colonization of implants was completely established and was stable after 2 weeks (Quirynen, Vogels et al. 2006; Furst, Salvi et al. 2007). Initial colonization of the microbiota on submucosal implant surfaces may occur within 10 to 14 days after implant installation and the microorganisms may be apparent in sulcular fluid (De Boever and De Boever 2006). *E. corrodens, F. nucleatum* subspecies *polymorphum, P. micros*, and *P. gingivalis* were increased in 8 weeks (van Winkelhoff, Goene et al. 2000). Between week 8 and 12, bacterial species included *P. gingivalis, T. forsythia*, and *T. denticola* were observed (Furst, Salvi et al. 2007).

The putative pathogenic bacteria can be found in healthy peri-implant tissue but in low frequency. Several cross-sectional studies have evaluated the complex microbial community growing on healthy peri-implant tissues and small amount of microorganisms with limited morphotypes, predominantly by gram-positive facultative bacteria, have been identified (Leonhardt, Renvert et al. 1999; De Boever and De Boever 2006; Furst,

Salvi et al. 2007). However, gram-negative anaerobic rods may also be found in small numbers and in low proportions with some dental implants. At the healthy implant sites, there was no apparent difference in the supra- or subgingival microbial groups (Listgarten 1976; Mombelli, van Oosten et al. 1987). Commensal oral bacterial species associated with healthy osseointegrated implants included *S. intermedius, S. oralis, S. sanguis, S. gordonii, V. parvular, F. nucleatum*, *C. gingivalis* (Mombelli, van Oosten et al. 1987; Rosenberg, Torosian et al. 1991; Lee, Maiden et al. 1999; Shibli, Melo et al. 2008), *A. naeslundii* type 1*, and S. mitis* (Shibli, Melo et al. 2008). *F. periodonticum,* a putative periodontal pathogen, presented supragingivally at the healthy sites (Shibli, Melo et al. 2008). In contrast, higher number and more diversity of oral bacteria were associated with infected implant sites.

Complications of dental implants may be observed in the early or late stages after implants placement. Early failures were frequently attributed to factors that interrupted osseointegration such as improper surgical technique or premature loading (Albrektsson 1983) whereas common complications after osseointegrated dental implants in function was the loss of crestal bone (Parr, Steflik et al. 1988). Bacterial infection was one of the contributing factors for implant failure after osseointegration (Becker, Becker et al. 1990; Rosenberg, Torosian et al. 1991).

Tissue responses around dental implants were dictated by transmucosal designs (Kim, Oh et al. 2010), while the designs and tissue response apparently

influenced the microbial ecosystem around dental implant (Canullo, Quaranta et al. 2010). The colonization pattern of the microbiota may predict the development of peri-implant lesions (Leonhardt, Renvert et al. 1999).

The composition of bacteria colonized in peri-implant lesion may be different when compared with healthy peri-implant sites. Peri-implantitis is referred to the peri-implant tissue inflammation with some bone loss and have been associated with a predominantly gram-negative anaerobic bacteria (Mombelli and Lang 1998). Clinical manifestation showed inflammatory reaction of the peri-implant mucosa with bleeding on probing and suppuration with osseous defects (Mombelli and Lang 1998; Klinge, Hultin et al. 2005; Lindhe and Meyle 2008). High plaque Index scores and total anaerobic bacteria counts with increased number of red complex species were associated with peri-implantitis affected subperiosteal implants but proportions of gram-positive facultative species shifted lower (Rams, Balkin et al. 2011). Numbers of *T. forsythia, P. gingivalis, T. denticola, F. nucleatum* subspecies *nucleatum, F. nucleatum* subspecies *vicentii* and *P. intermedia* increased at the diseased implants sites (Shibli, Melo et al. 2008). Peri-implantitis lesions were also reported to contain staphylococci, enteric species, and yeast (Leonhardt, Renvert et al. 1999) suggesting the concepts that infections of tissue surrounding implants is associated with more complex microbiota (Renvert, Roos-Jansaker et al. 2007).

The composition of the peri-implant microbiome may be the important determinants of the long-term clinical status of dental implants. Moreover, the treatment of implant failures should not be achieved without an effective microbiological analysis (Alcoforado, Rams et al. 1991). Nonetheless, the specific bacteria associating with different transmucosal designs are poorly understood.

CHAPTER III

METHODOLOGY

Sample collection

Patients, who received implant-supported fixed partial prostheses from Esthetic Restorative and Implant Department, and Special Clinic, Faculty of Dentistry, Chulalongkorn University, were examined based on the inclusion and exclusion criteria (Table 1). Forty-five participants were randomly included in this study. All participants were in good health. Each exclusion criteria was conducted in order to eliminate either the factors that influence post-loading marginal bone loss or changing of microbial composition around implant-abutment interface. The steps in sample collection were thoroughly explained to all subjects. The informed consent included the possible risks and benefits of participation in the study. All subjects were required to sign a consent form in order to participate in this study, and the subjects were free to withdraw from the study at any time.

Table 1. Inclusion and Exclusion Criteria

Inclusion criteria	Exclusion criteria
- Patients who have 1-3 dental implants supporting fixed partial prostheses - Patients who have no acute infection at any implant sites - Patients who received implant-supported restorations for at least 1 year - Patients who have no interproximal or buccal bone defect at implant sites - Patients who have no visible inflammed tissue - Patients who are non-smoker	- Patients who have generalized or localized periodontitis which is defined as presence of any site with peri-implant pocket depth more than 4 mm and attachment loss more than 2 mm. (define term: peri-implant pocket and attachment loss) - Patients have any infection at the adjacent natural teeth - Patients have parafunctional habits - Patients with uncontrolled systemic diseases such as uncontrolled diabetes - Patients who have continued antibiotics for 3 months - Patients who were pregnant or lactating

Subjects were divided into 3 groups according to transmucosal designs, including group I, implants restored with matching diameter abutments, group II, implants restored with platform-switching abutments, and group III, tissue-level implants, as appearing on periapical radiographs. To our knowledge, there is little evidence to

compare the putative periodontal pathogens around implant tissue in each specific platform.

Oral hygiene was assessed and scored as good, fair, and poor depending on the accumulation of bacterial plaque and/or calculus by inspection. The sites were isolated with sterile gauze and the supragingival plaque and calculus was removed with plastic curette in order to avoid contamination when the subgingival crevicular fluid was collected.

Periodontal parameters such as bleeding on probing and probing depth were assessed. The probing depth was measured on the mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual aspects of each implant by plastic periodontal probe (Periowise; Premier Dental, Playmouth Meeting, PA) (Fig. 2). Periapical radiographs were taken to measure the distance from the mesial and distal margins of the implant neck to the most coronal point where the bone appeared to be in contact with the implant.

Four paper points were inserted under light pressure in the peri-implant sulcus that presented the deepest probing depths until resistance was felt and were kept there for 10 seconds (Slots, Ashimoto et al. 1995) (Fig. 3) to obtain peri-implant subgingival crevicular fluid. After this step, these paper points were cut with sterile scissor and forceps to 5 mm length and kept in labeled plastic collection tube (Fig. 4). Then, each plastic collection tube was stored at -20°C in a freezer until processing.

Figure 2. Plastic periodontal probe

Figure 3. Peri-implant subgingival fluid collection

Figure 4. 1.5 ml Collection Tube

DNA extraction

DNA was extracted using PowerBiofilm™ DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA USA) according to the manufacturer's instruction. Paper points in each plastic collection tube were washed with 350 µl BF1 (warmed at 55ºc for 10 minutes before used) then were transferred to bead tube. 100 µl BF2 was added and mixed using a vortex mixer. All the bead tubes were incubated at 65ºc for 5 minutes. After the incubation, all samples were beaded using Vortex Adapter at maximum speed for 10 minutes followed by centrifugation at 13,000 rcf for 1 minute at room temperature. Then, the supernatant was transferred to 2 ml collection tube. 100 µl

BF3 was added and mixed using a vortex mixer. All samples were incubated at 4ºc on ice for 5 minutes follow by centrifugation at 13,000 rcf for 1 minute at room temperature. Thereafter, the entire supernatant was transferred to 2 ml collection tube avoiding pellet. 900 μl BF4 was added and mixed using a vortex mixer. 650 μl of the supernatant was loaded onto spin filter and centrifuged at 13,000 rcf for 1 minute at room temperature. Then, discarding the flow through and repeating this step until the entire supernatant was loaded. After this step, the spin filter basket was placed into 2 ml collection tube. 650 μl BF5 was added followed by centrifugation at 13,000 rcf for 1 minute at room temperature and discarding the flow through. 650 μl BF6 was added followed by centrifugation at 13,000 rcf for 1 minute at room temperature and discarding the flow through. The centrifugation was repeated at 13,000 rcf for 2 minute at room temperature to remove the residual wash. Afterward, the spin filter basket was placed into the new 2 ml collection tube. 50 μl BF7 was added to the center of the white filter membrane and all samples were stored for 5 minutes at room temperature followed by centrifugation at 13,000 rcf for 1 minute at room temperature, and then the spin filter basket was discarded. This procedure was followed by quantification the DNA samples using NanoDrop2000 Spectrophotometer (Thermo SCIENTIFIC, Wilmington DE, USA) (Fig. 5).

Figure 5. DNA quantification by NanoDrop2000 Spectrophotometer

Microbial Identification by PCR

Endpoint PCR was used to detect periodontal pathogens by using bacterial-specific primers. Sequences of bacterial-specific primers show in Table 2. 1µl of DNA (50 ng) was mixed with 12.5µl of TopTaq Master Mix (TopTaq DNA Polymerase, dNTPs, and the innovative TopTaq PCR Buffer), 1µl of forward species-specific primer, 1µl of reverse species-specific primer, and the PCR water was added to total volume of 25 µl. The PCR reaction was carried out by using S1000 Thermal Cycler (Fig. 6). The program

consisted of an initial step at 94°C for 3 min, and amplifications performed for 35 cycles, with denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 60 s, followed by extension at 72°C for 10 min. Reaction products were electrophoresed (Fig. 7) in 1.5% agarose gels, stained with ethidium bromide and photographed under ultraviolet light. A 100 base pair DNA Ladder (Invitrogen Corp.Carlsbad, CA) was used as base pair marker.

Table 2. Sequences of bacterial-specific primers

Primer	5' → 3' Sequences
P.gingivalis (forward)	AGGCAGCTTGCCATACTGCG
P.gingivalis (reverse)	ACTGTTAGCAACTACCGATGT
T.forsythus (forward)	GCGTATGTAACCTGCCCGCA
T.forsythus (reverse)	TGCTTCAGTGTCAGTTATACCT
T.denticola (forward)	TAATACCGAATGTGCTCATTTACAT
T.denticola (reverse)	TCAAAGAAGCATTCCCTCTTCTTCTTA

Figure. 6 S1000 Thermal cycler
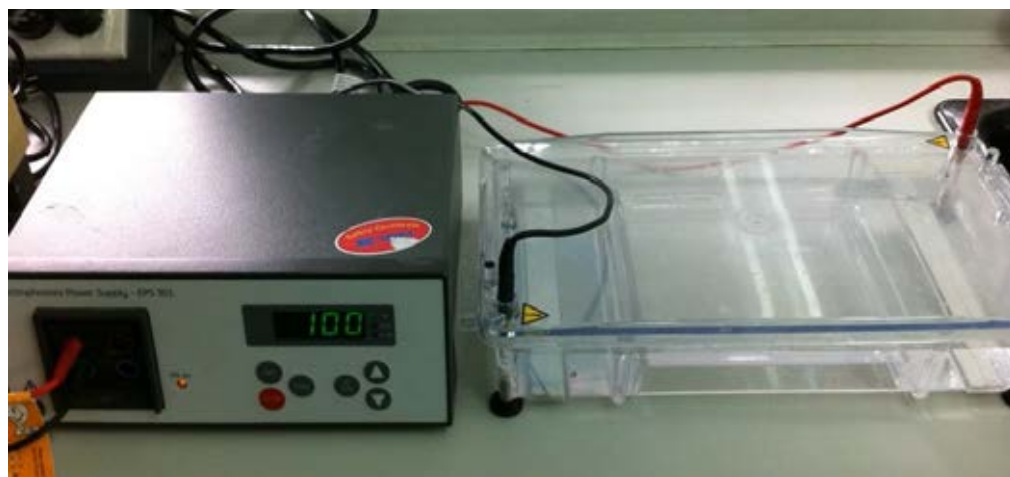
Figure. 7 DNA Gel Electrophoresis

Statistical analysis

All data were analyzed using SPSS program (SPSS version 16.0, SPSS Inc., Chicago, IL). The chi-square test was used to analyze the presence of each bacterium (*P. gingivalis*, *T. forsythia*, and *T. denticola*) within each group and to compare the presence of all bacteria among groups. The percentage of subjects colonized by at least one bacterium was calculated as followed: [(number of subjects colonized by at least one bacterium/total number of subjects from the same group) x 100].

CHAPTER IV

RESULTS

The demographic data of the study population are summarized in Table 3. The mean ages of the subjects in the dental implant restored with platform-matching abutments (group I), dental implant restored with platform-switching abutments (group II), and tissue level dental implants (group III) were 58.93, 47.2, and 53.07 years, respectively. However, the mean of loading years was high in group I since the platform-matching design has been routinely used for the treatment for years earlier than other designs. All implant sites appeared to be healthy and tissue sulci around dental implants were approximately 3 to 4 mm as shown by probing depth.

Table 3. Summary of demographic and clinical results in each group

	Group I	Group II	Group III
Age* (years)	58.93±9.49	47.2±10.53	53.07±6.37
Gender (M/F)	9/6	2/13	8/7
Loading* (years)	4.5±3.13	1.68±0.46	2.07±1.00
Probing depths* (millimeter)	3.6±0.51	3.2±0.63	3.4±0.69

* Mean±SD were shown

The prevalence of each periodontal pathogen in subgingival healthy peri-implant sites for each transmucosal abutment design was shown in Table 4. Chi-square analysis

demonstrated no significant difference (P > 0.05) in the frequencies of *P. gingivalis*, *T. forsythia*, and *T. denticola* among the comparison groups. The frequencies of the red complex bacteria were significant difference (P < 0.05) in the implants restored with matching abutments (group I) and platform-switching abutments (group II), but not tissue-level dental implants (group III).

Table 4. Frequency of bacteria detected by end-point PCR

Frequency of Bacteria				
Group (n)	*P. gingivalis*	*T. forsythia*	*T. denticola*	p-value
Group I (n = 15)	1	8	3	0.004
Group II (n = 15)	1	9	2	0.000
Group III (n = 15)	1	7	3	0.087
p-value	1.000	0.765	0.859	

Among the red complex bacteria, *T. forsythia* was the most detectable in the healthy peri-implant pockets (Table 4 and 5). We found that *P. gingivalis* was present only in samples positive for *T. denticola* or *T. forsythia* (Table 5). *T. denticola* was presented at low frequency regardless of the transmucosal designs. Most of *T. denticola*–positive samples were also positive for other red complex bacteria (Table 5).

Table 5.The number of subjects colonized by *Pg, Tf* or *Td* individually, or

in combinations

Group (n)	Number of subjects colonized by one bacterium or combinations						Percentage of Subjects Colonized by at least one bacterium
	Pg	*Tf*	*Td*	*Pg-Td*	*Tf - Td*	*Pg - Tf - Td*	
Group I (15)	0	6	1	0	1	1	60%
Group II (15)	0	8	0	1	1	0	66.67%
Group III (15)	0	4	0	0	2	1	46.67%

CHAPTER V

DISCUSSION, CONCLUSION AND RECCOMMENDATIONS

Discussion

All patients in this study were partially edentulous and restored with fixed partial dentures in order to eliminate the different biological and technical complications as reported in systematic review of Berglundh et al. (Berglundh, Persson et al. 2002). The variation of the bacterial microbiota and the proportion of periodontal pathogens were more pronounced in healthy partially edentulous patients than in edentulous patients (Mombelli 1993). Healthy peri-implant sites maintained shallow tissue pockets with aerobic or facultative condition. The anerobic, putative periodontal pathogens, *P. gingivalis*, *T. forsythia*, and *T. denticola,* or a so-called 'red complex' were strongly associated with periodontal disease and tissue inflammation (Leonhardt, Renvert et al. 1999; Ledder, Gilbert et al. 2007) (Hosaka, Saito et al. 1994; Shibli, Melo et al. 2008). Although, the red complex bacteria were significantly associated with periodontal disease (Hosaka, Saito et al. 1994; Shibli, Melo et al. 2008), they may be occasionally present in healthy implant sites (Gmur, Strub et al. 1989). The study of Botero et al. reported that *P. gingivalis* was not present with healthy implants using culture-based method (Botero, Gonzalez et al. 2005). However, these anaerobic bacteria were sometimes difficult to be detected by culture-based method. The molecular method,

such as PCR, was more sensitive. This study and the study of Leonhardt A et al. suggested that these pathogens are part of the normal resident microbiota and may be found at both stable and progressing inflammatory sites (Leonhardt, Grondahl et al. 2002).

To understand the influence of abutment transmucosal designs to periodontal pathogen colonization, we investigated the prevalence of *P. gingivalis*, *T. forsythia*, and *T. denticola* in subgingival healthy peri-implant sites by PCR. The mean ages of all subjects in this study were approximately 53.06 years, which represented the similar mean age of each study group. The mean of loading years in Group I was high, because the platform-matching design has been routinely used for the treatment for years earlier than other designs. All implant sites appeared to be healthy and tissue sulci around dental implants in physiologic range as shown by probing depth. Based on our results, the frequencies of all three bacteria were statistically significant different ($P < 0.05$) in the implants restored with platform-matching (Group I) and platform-switching (Group II), but not in the tissue- level dental implants (Group III). These results suggested that the transmucosal design might influence the colonization of the red complex bacteria. Since the position of the abutment connection may reflect to depth of peri-implant tissue, this result suggested that the coronal position of the microgap at the component connection might be important for predicting putative pathogen colonization.

Periodontal tissue inflammation is associated with the breakdown of host-microbial homeostasis, which caused by multispecies bacteria rather than a single pathogen (Marsh 1994). *P. gingivalis* and *T. denticola* was presented at low frequency regardless of the transmucosal designs, but *T. forsythia* seemed to be detected at high frequency. That might not be associated with periodontal destruction (Gmur, Strub et al. 1989). Interestingly, *P. gingivalis* and *T. denticola* was mostly detected in combination with other red complex bacteria (Table 2). The study of Gmür, R et al. demonstrated that both *P. gingivalis* and *T. forsythia* were present in shallow pocket, and the proportion of *P. gingivalis* to *T. forsythia* was increased corresponding to the depth of periodontal pocket. Recent studies have shown synergistic growth of *T. denticola* in *in vitro* coculture with *P. gingivalis* (Nilius, Spencer et al. 1993), and the simultaneous presence of *P. gingivalis*, *T. forsythia*, and *T. denticola* was strongly associated to adult periodontitis (Kasuga, Ishihara et al. 2000). Thus, the site-specific microbiota could play a role in the microenvironment such as the peri-implant tissue depths.

Platform-switching design appeared to have similar frequencies of putative pathogens to the platform-matching design, whereas tissue level dental implants demonstrated fewer putative pathogens. None of the red complex bacteria were detected in 53.33% of subjects with tissue level design (Table 3) regardless of age or depth of surrounding tissue pockets. Consistent to the previous report (Hermann, Buser et al. 2001), our data suggested that the microgap was the important factor associated

with the bacterial colonization position. We found that the tissue level implant was likely to have less richness of the red complex bacteria. Whether the increase of the red complex bacteria in bone level implants would influence soft tissue and bone remodeling is to be further investigated.

It was clearly shown that the bone level, platform-switching design shifted the microgap inward horizontally gained biomechanical benefits for stress control (Canullo, Pace et al. 2011) and esthetics (Buser 2010). For a long-term maintenance, however, it is not yet clear whether the tissue-level design has more advantage. Platform-switching design may be better than tissue-level design because of crestal bone preservation. The putative periodontal pathogens do not lead to periodontal tissue breakdown in short period of time (Casado, Otazu et al.). Yet, the change of clinical conditions after years in function such as the excessive occlusal force in addition to bacterial infection may be the cause of peri-implant tissue inflammation and compromise the success of dental implants.

Conclusions

The putative periodontal pathogens may present at the healthy dental implant sites regardless of transmucosal designs. The prevalence of the red complex bacteria was not affected when the microgap is inwardly positioned in the platform-switching design. However, the coronal position of the microgap in the tissue level implant showed

more favorable results on the bacterial colonization. In healthy condition, the low level of

putative pathogen colonization is clinically insignificant, but pathologic shift may occur in

response to other environmental changes.

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Appendix

The presence of pathogenic bacteria in group I

	Pg	*Tf*	*Td*
Sample 1	0	1	1
Sample 2	0	1	0
Sample 3	0	1	0
Sample 4	0	0	0
Sample 5	0	0	0
Sample 6	0	1	0
Sample 7	0	0	0
Sample 8	0	1	0
Sample 9	0	1	0
Sample 10	0	0	0
Sample 11	0	0	0
Sample 12	0	0	0
Sample 13	1	1	1
Sample 14	0	1	0
Sample 15	0	0	1

0 = not exist, 1 = exist

The presence of pathogenic bacteria in group II

	Pg	Tf	Td
Sample 1	0	1	0
Sample 2	0	0	0
Sample 3	0	0	0
Sample 4	0	1	0
Sample 5	0	1	0
Sample 6	0	1	1
Sample 7	0	1	0
Sample 8	0	1	0
Sample 9	0	1	0
Sample 10	0	0	0
Sample 11	0	0	0
Sample 12	0	1	0
Sample 13	0	1	0
Sample 14	0	0	0
Sample 15	1	0	1

0 = not exist, 1 = exist

The presence of pathogenic bacteria in group II

	Pg	Tf	Td
Sample 1	0	1	1
Sample 2	0	1	0
Sample 3	0	0	0
Sample 4	0	0	0
Sample 5	0	0	0
Sample 6	0	1	1
Sample 7	0	0	0
Sample 8	0	0	0
Sample 9	0	0	0
Sample 10	0	1	0
Sample 11	0	0	0
Sample 12	0	1	0
Sample 13	0	1	0
Sample 14	0	0	0
Sample 15	1	1	1

0 = not exist, 1 = exist

Chi Square test of the detection frequency of *P. gingivalis* among groups

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
abutment * Pg	45	100.0%	0	.0%	45	100.0%

abutment * Pg Crosstabulation

			Pg		
			no	yes	Total
abutment	matching abutment	Count	14	1	15
		Expected Count	14.0	1.0	15.0
	switching platform	Count	14	1	15
		Expected Count	14.0	1.0	15.0
	tissue level	Count	14	1	15
		Expected Count	14.0	1.0	15.0
Total		Count	42	3	45
		Expected Count	42.0	3.0	45.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.000[a]	2	1.000
Likelihood Ratio	.000	2	1.000
Linear-by-Linear Association	.000	1	1.000
N of Valid Cases	45		

a. 3 cells (50.0%) have expected count less than 5. The minimum
expected count is 1.00.

Chi Square test of the detection frequency of *T. forsythia* among groups

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
abutment * Tf	45	100.0%	0	.0%	45	100.0%

abutment * Tf Crosstabulation

			Tf		Total
			no	yes	
abutment	matching abutment	Count	7	8	15
		Expected Count	7.0	8.0	15.0
	switching platform	Count	6	9	15
		Expected Count	7.0	8.0	15.0
	tissue level	Count	8	7	15
		Expected Count	7.0	8.0	15.0
Total		Count	21	24	45
		Expected Count	21.0	24.0	45.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.536[a]	2	.765
Likelihood Ratio	.537	2	.764
Linear-by-Linear Association	.131	1	.717
N of Valid Cases	45		

a. 0 cells (.0%) have expected count less than 5. The minimum
expected count is 7.00.

Chi Square test of the detection frequency of *T. denticola* among groups

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
abutment * Td	45	100.0%	0	.0%	45	100.0%

abutment * Td Crosstabulation

			Td		Total
			no	yes	
abutment	matching abutment	Count	12	3	15
		Expected Count	12.3	2.7	15.0
	switching platform	Count	13	2	15
		Expected Count	12.3	2.7	15.0
	tissue level	Count	12	3	15
		Expected Count	12.3	2.7	15.0
Total		Count	37	8	45
		Expected Count	37.0	8.0	45.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.304[a]	2	.859
Likelihood Ratio	.316	2	.854
Linear-by-Linear Association	.000	1	1.000
N of Valid Cases	45		

a. 3 cells (50.0%) have expected count less than 5. The minimum
expected count is 2.67.

Chi square test of the detection frequency of each bacterium within group I

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
micro * exist	44	97.8%	1	2.2%	45	100.0%

micro * exist Crosstabulation

			exist		Total
			0	1	
micro	Pg	Count	14	1	15
		Expected Count	11.2	3.8	15.0
	Td	Count	12	2	14
		Expected Count	10.5	3.5	14.0
	Tf	Count	7	8	15
		Expected Count	11.2	3.8	15.0
Total		Count	33	11	44
		Expected Count	33.0	11.0	44.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.968[a]	2	.007
Likelihood Ratio	9.927	2	.007
N of Valid Cases	44		

a. 3 cells (50.0%) have expected count less than 5. The minimum
expected count is 3.50.

Chi square test of the detection frequency of each bacterium within group II

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
micro * exist	45	100.0%	0	.0%	45	100.0%

micro * exist Crosstabulation

			exist		Total
			0	1	
micro	Pg	Count	15	0	15
		Expected Count	11.3	3.7	15.0
	Td	Count	13	2	15
		Expected Count	11.3	3.7	15.0
	Tf	Count	6	9	15
		Expected Count	11.3	3.7	15.0
Total		Count	34	11	45
		Expected Count	34.0	11.0	45.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	16.123[a]	2	.000
Likelihood Ratio	18.083	2	.000
N of Valid Cases	45		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 3.67.

Chi square test of the detection frequency of each bacterium within group III

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
micro * exist	45	100.0%	0	.0%	45	100.0%

micro * exist Crosstabulation

			exist		
			0	1	Total
micro	Pg	Count	14	1	15
		Expected Count	11.7	3.3	15.0
	Td	Count	12	3	15
		Expected Count	11.7	3.3	15.0
	Tf	Count	9	6	15
		Expected Count	11.7	3.3	15.0
Total		Count	35	10	45
		Expected Count	35.0	10.0	45.0

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.886[a]	2	.087
Likelihood Ratio	5.123	2	.077
N of Valid Cases	45		

a. 3 cells (50.0%) have expected count less than 5. The minimum
expected count is 3.33.

BIOGRAPHY

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