BACTERIAL COLONIZATION OF THE DENTAL IMPLANT FIXTURE-ABUTMENT INTERFACE

By

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To my daughter, Mary Katherine
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I would like to thank my family, who has supported me throughout all my endeavors without hesitation. Additionally, I would like to extend my gratitude to the faculty members of the University of Florida department of Periodontology for their contribution to my education and their continuing commitment to our profession.
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The geometry of the fixture–abutment interface (FAI) might influence the risk of bacterial invasion of the internal part of the implant. The aim of this study was to use an in vitro model to assess the potential risk for invasion of oral microorganisms into the FAI microgap of dental implants with different characteristics of the connection between the fixture and abutment.

Thirty implants were divided into three groups (n = 10 per group) based on their microgap dynamics. Groups 1 and 2 were comprised of fixtures with internal Morse taper connections that connected to standard abutments and the same abutments with a 0.5-mm groove modification, respectively. Group 3 was comprised of implants with a tri-channel internal connection. Fixtures and abutments were assembled and allowed to incubate in a bacterial solution of \textit{Aggregatibacter actinomycetemcomitans} (previously \textit{Actinobacillus actinomycetemcomitans}) and \textit{Porphyromonas gingivalis}; chosen based upon the knowledge of their presence in disease of both the periodontal and peri-implant tissues. Two standard abutments were either exposed to bacterial culture or left sterile to serve as positive and negative controls. After disconnection of fixtures and abutments, microbial samples were taken from the threaded portion of the abutment.
using sterile calcium alginate swabs, plated directly, and allowed to culture under appropriate conditions.

Three of the 10 samples in group 1 developed one colony forming unit (CFU) for A. *actinomycetemcomitans*, whereas zero of 10 samples developed CFUs for P. *gingivalis*. Ten of 10 and nine of 10 samples from groups 2 and 3, respectively, developed multiple CFUs for A. *actinomycetemcomitans* and P. *gingivalis*.

This study indicated that differences in implant designs may affect the potential risk for invasion of oral microorganisms into the FAI microgap.
CHAPTER 1
INTRODUCTION

The quantity and quality of the bone surrounding a dental implant influences implant osseointegration and affects the shape and contour of the overlying soft tissues and, consequently, the esthetic outcome. Only with careful considerations of the biologic principles of peri-implant soft and hard tissues, as well as the appropriate selection of implant type and position, can a functional and esthetic treatment result be achieved.\(^1,2\) Early bacterial colonization around implants by microorganisms associated with periodontitis has been reported\(^3-5\), and this colonization of implant surfaces and peri-implant tissues can occur within minutes after implant placement.\(^6\)

When a prosthetic abutment is connected to a fixture, a microgap is created between the components. Microorganisms may grow into this fixture–abutment interface (FAI) microgap\(^7-9\) and set up a bacterial reservoir, resulting in an area of inflamed soft tissue facing the fixture–abutment junction.\(^10\) A study by Callan et al.\(^9\) used DNA probe analysis to examine the bacterial colonization into the FAI in patients. The authors reported moderate to high levels of eight different putative periodontal pathogens, including *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*) and *Porphyromonas gingivalis*, colonizing the FAI. These findings support the results of other researchers\(^4,5\) indicating that normal and pathogenic oral microflora was able to penetrate and colonize the implant abutment interface of dental implants.

Thus, the presence of an FAI microgap in close relation to bone may have a role in the development of peri-implant inflammation and bone loss.\(^11-16\) Furthermore, when using one-piece implants that do not have an FAI microgap, minimal early bone re-
sorption was found. This result is consistent with the favorable 8-year outcomes of one-piece implants in patients reported by Buser et al.

The design of the FAI may have an impact on the amount of microbial penetration into the internal part of dental implants. For instance, in an in vitro study, Quirynen et al. demonstrated the microbial penetration of the FAI microgap of fixtures with an external hex design. However, there was no comparison among implants with different FAI designs in the study. Jansen et al. reported microbial leakage of 13 different implant–abutment combinations using Escherichia coli as indicator bacteria. Among the different implant–abutment combinations, an implant with an internal connection and a silicon washer demonstrated the fewest cases of leakage. In the report by Callan et al., implants from different manufacturers were used without the authors specifying the characteristics of the FAI geometry. Therefore, despite the fact that they reported moderate to high levels of colonization of the FAI microgap by periodontal pathogens, it was not possible to evaluate the impact of the design of the FAI on the microbial penetration. Thus, there is limited information regarding differences in the microbial penetration of the FAI microgap of implants with different internal connection designs.

Hypothesis: It is hypothesized for this study that there is no difference in potential invasion of oral microorganisms into the FAI microgap in dental implants with different internal connection designs.
CHAPTER 2
BACKGROUND

History of Osseointegration and Root Form Implants in Restorative Dentistry

The successful replacement of missing natural teeth by tissue-integrated root form dental implants has been a major advance in clinical dental treatment. The science behind the osseointegration method has evolved over the past several decades in both laboratory and clinical settings. In the early 1950s bone already had been observed to attach to titanium and was well tolerated as an implant material by various tissues in animal experiments\textsuperscript{19, 20}. Still, in the early 1960s and 1970s the idea of the metal implant was far from accepted as a biocompatible material.\textsuperscript{21} Dr. Per-Ingvar Brånemark has been accredited with the early research done in the 1960s that eventually evolved into today's modern root form implants. Although the initial research concept leading to modern day implant osseointegration has been thought of as a chance occurrence. It is without doubt that Brånemark’s careful attention to detail and meticulous efforts was what laid down the foundation for what some have characterized as a shift in paradigm concerning dental implants.\textsuperscript{22} Many different types of implant systems have been used to replace missing teeth, including subperiosteal implants (Figure 2-1)\textsuperscript{23} and blade implants (Figure 2-2).\textsuperscript{24} However, not until 1983 with the widespread introduction of the endosteal osseointegrated dental implant\textsuperscript{25}, that dental implants began to gain a wider acceptance as a replacement for natural teeth and as prosthesis support. In the report on osseointegration of dental implants by Brånemark, the indications for treatment were limited to the edentulous arch.\textsuperscript{26} In more recent times the osseointegrated dental implant has successfully become more widely used in partially edentulous patients, as a single tooth replacement.\textsuperscript{27-29}
Figure 2-1. Example of the subperiosteal dental implant. (source: University of Connecticut Health Center. http://dentalimplants.uchc.edu/about/types.html. last accessed January 2011)

Figure 2-2. Examples endosseous osseointegrated dental implants: blade and root form. (source: University of Connecticut Health Center. http://dentalimplants.uchc.edu/about/types.html. last accessed January 2011)
The term osseointegration was originally proposed in 1977 in the 10-year report by Brånemark et al. 1977. This concept had been previously outlined in 1969 in an animal study on the experimental use of intra-osseous retention of dental prosthesis. This term was defined by Albrektsson as “a direct functional and structural connection between living bone and the surface of a load carrying implant”. This definition was historically a histologic concept at the level of the light microscope. As this was not a practical or clinically applicable definition; this phenomenon has been defined by several authors from various levels of observation and viewpoints. A new definition based on implant stability was suggested by Zarb & Albrektsson as a process whereby clinically asymptomatic rigid fixation of alloplastic materials is achieved and maintained in bone during functional loading. Schroeder later used the term “functional ankylosis” to describe the integration of the fixture into the alveolar bone. The course of osseointegration is achieved through a number of biologic processes, which has been described by Brånemark et al. (1969) and Schroeder et al. (1976). More recently, a study by Berglundh et al. evaluated osseointegration patterns in animals at varying time intervals. A U-shaped circumferential trough had been prepared within the thread region of the implants (intraosseous portion), but leaving the tip of each pitch untouched. Hereby, a secluded area, an experimental wound chamber, was created following implant installation. The authors observed that immediately after the fixture is placed into the osteotomy, a blood clot formed and soon became surrounded by inflammatory cells (Figure 2-3). The peripheral portions of the pitches of the thread remained in close contact with surrounding bone providing mechanical stability during the initial phase of wound healing. Days later the coagulum is replaced by granulation
tissue containing the cells needed to form provisional connective tissue. In this newly formed tissue immediately lateral to the titanium surface, the densely packed cells resided in a stroma of fibrin-like structures where only a few inflammatory cells are still present (Figure 2-4). As weeks pass an immature woven bone is seen surrounding the implant. This newly formed bone was seen to occupy almost all surface regions of the implant. Bone tissue next to the implant wall was lined with osteoblasts facing a provisional matrix rich in vascularity (Figure 2-5). At six weeks large areas of newly formed bone were characterized by the occurrence of primary and secondary osteons, and mineralized tissues were in close contact with the implant surface. After 8 to 12 weeks, noted signs of remodeling could be seen. Mineralized hard tissue was surrounded by bone marrow, containing adipocytes, vessels, collagen fibers and some mononuclear leukocytes of the mature lamellar bone as the osseointegration process completes at around 3 months (Figure 2-6).  

Osseointegration represents a dynamic process during both its establishment and its maintenance. In the establishment phase, there is a delicate interplay between bone resorption in contact regions between the titanium implant body and mineralized bone, and bone formation in ‘contact-free’ areas. During the maintenance phase, osseointegration is secured through continuous remodeling and adaptation to function. The patterns of bone formation observed in the osseointegration model described in the previous study by Berglundh et al. are also consistent with previous descriptions of bone modeling and remodeling in bone defects of varying locations and dimensions, including extraction sockets. However, it should be realized that the size and
configuration of the wound defect to undergo bone modeling and remodeling will influence the rate of completion of the healing process.\textsuperscript{37}

**Evaluation of Dental Implants: Clinical and Radiographic Parameters**

Long-term follow-up studies are corner stones in clinical evaluations of medical and dental treatment modalities. In the field of dental implants, Adell and co-workers presented two classical long-term follow-up studies\textsuperscript{27,38} that have been used to validate the use of osseointegrated implants to rehabilitate edentulous patients. In their study from 1990 they found that 95% of the maxillae and 99% of the mandibles had continuous prosthesis stability after 15 years. Since then, numerous follow-up studies

![Series of histologic slides after dental implant placement](image)

**Figure 2-3.** Series of histologic slides after dental implant placement (a) Implant with surrounding soft and hard tissues sampled 2 h after installation, ground section, original mag. x 16, wound chambers created between the pitches of the thread (b) Pitches (arrows) in close contact with the bone tissue, wound chamber filled with coagulum, ground section, original mag. x 50 (c) Wound chamber with coagulum 2 h after device installation, decalcified section, original mag. x 100 (d) Coagulum including large numbers of erythrocytes and some inflammatory cells, original mag. x 400.\textsuperscript{38}
Figure 2-4. Implant wound chamber filled with a tissue in close contact with the SLA surface representing 4 days of healing, ground section. original mag. x 100.\textsuperscript{35}

Figure 2-5. Implant wound chamber representing 4 weeks of healing, original mag. x 100, portions of the mineralized part of the primary spongiosa are in apparent contact with the SLA surface.\textsuperscript{35}
Figure 2-6. After 12 weeks the chamber is occupied with mature bone and includes also areas of bone marrow in contact with the implant surface (ground section; original mag. x 100).\(^{35}\)

on dental implants have been published, and today several studies cover 10 years or more for different patient categories.\(^{39-46}\)

Although dental implant therapy is regarded as a safe and reliable procedure, complications do occur. Many authors have discussed different factors that may cause failures in implant treatment, but most likely implant failures have a multi-factorial background.\(^{47}\) Esposito et al. 1998 divided implant failures into four groups; biological failures (related to the biological process), mechanical failures of the components (fractures of implants, connecting screws, coatings and prostheses), iatrogenic failures (e.g. nerve damage, wrong alignment of the implant), and functional failures (phonetical, aesthetical, psychological problems). Further, they classified the biological failures as endogenous (systemic and local) and exogenous (operator- and biomaterial-related).\(^{32}\) Later, Esposito and co-workers (1999) defined biological failures as “the inadequacy of the host to establish or to maintain osseointegration”. When an implant does not
become osseointegrated it can be regarded as an early failure, in contrast to a late failure resulting from the loss of an achieved osseointegration under functional conditions. Berglundh et al. described other incidences of biological and technical complications in a (2002) meta analysis. This review included incidences based upon: Implants lost before loading, implants lost during function, persisting sensory disturbance, soft tissue complications requiring therapy, peri-implantitis, crestal bone loss, implant fracture, complications related to implant components, and complications related to suprastructures. The authors concluded that there is limited information on the incidence of peri-implant infections such as peri-implantitis as well as the occurrence of crestal bone loss. This information was due to the lack of data describing clinical parameters associated with such incidences.

There are several methods used to evaluate dental implants. Some methods mainly focus in evaluating the stability of the dental implant that reflects the status of osseointegration and others focusing on evaluation of the peri-implant mucosa that reflects status of health of the soft tissues surrounding the implant. Methods for evaluating the peri-implant tissues include examination and record of: bleeding on probing, suppuration, as well as probing depth measurements relating to marginal bone levels. In addition to those methods, radiographic examination can provide further information regarding the bone topography around a dental implant.

**Evaluation of Osteointegration:**

There are various methods used to evaluate whether osseointegration has taken place or not. A simple method is to test implant stability by exerting a clockwise force on the abutment with an implant driver. The implant can be considered osseointegrated if the implant is found to be immobile. Another method is to tap the abutment with a
metallic instrument. A high-pitched metallic sound will then indicate an integrated implant.\textsuperscript{51}

A number of authors\textsuperscript{52-54} have reported the potential application of the Periotest in measuring implant mobility. The Periotest device (Siemens, Bensheim, Germany) is an electronic instrument originally designed for quantitative measurements of damping characteristics of the periodontal ligament to establish a numerical value for tooth mobility.\textsuperscript{55} The device is, however, operator sensitive and its value as a clinical diagnostic method to measure implant stability has been questioned.\textsuperscript{56-58}

Resonance frequency analysis (RFA) is another method, developed by Meredith et al. (1996), to evaluate implant stability and found to be of clinical value.\textsuperscript{59-61} A small beam-like transducer (Osstell, Integration, Diagnostics AB, Partille, Sweden) is attached to the implant or the abutment. The transducer can electronically make the implant to vibrate and the response is measured and registered. The technique is influenced both by the exposed length of the implant and the stiffness of the interface between the implant and the bone. Huwiler et al. (2007) found that even with a good initial implant stability, as measured with the RFA technique, implants might later on fail.\textsuperscript{62}

**Evaluation of Peri-Implant Mucosa:**

The examination of the peri-implant tissues around implants has many features in common with the periodontal examination. The clinical examination must, according to Lang & Lindhe (2003), include parameters such as bleeding on probing, probing depth, and suppuration.\textsuperscript{63} All these assessments can reveal whether the mucosa around the implant is healthy or not. When probing a pocket around an implant, surrounded by an unhealthy mucosa, the probe goes beyond the sulcus and reaches closer to the bone than it does around a tooth.\textsuperscript{64} Under healthy conditions the pocket depth, for
conventionally placed implants, ranges between 2-4 mm.\textsuperscript{65} Originally the value of peri-
implant probing in determining the status of the peri-implant tissues was questioned.\textsuperscript{66} However, in recent years the usefulness of the information derived from it has generally been accepted.\textsuperscript{67} Probing a pocket is often a difficult task since it is painful for the patient. Further, the assessed pocket depth depends on the pressure applied during probing which makes probing operator sensitive and unreliable. Lang and colleagues (2004) stated that peri-implant probing should be performed with a light force (\textit{i.e.} 0.2-0.25 N) to avoid tissue trauma.\textsuperscript{65} Lekholm et al. (1986) found the presence of deep pockets not to be associated with an accelerated marginal bone loss.\textsuperscript{68} Clinical probing depths and radiographic bone levels have been compared to histological bone levels around screw type implants in monkeys. The radiographic bone level was on average 0.1-0.5 mm, depending on type of implant, short of the histological bone level. The corresponding value for the probing level was much higher, 1.1-3.9 mm.\textsuperscript{69}

It has been established that bleeding on probing is a valuable parameter in assessing the health status of periodontal tissues.\textsuperscript{70,71} In particular, the absence of bleeding on probing has been shown to be a predictor of periodontal stability.\textsuperscript{72} The size of the tip of the probe as well as the probing force should be standardized to obtain meaningful data.\textsuperscript{73,74}

Studies comparing bleeding scores at teeth and implants in the same mouth have reported that the bleeding on probing frequencies are higher at implants compared to teeth.\textsuperscript{67} This evidence has been further supported by a (2008) review by Heitz-Mayfield et al. where the authors concluded that there is evidence that probing using a light force (0.25N) does not damage the peri-implant tissues and that bleeding on probing
indicates presence of inflammation in the peri-implant mucosa. The authors also stated that probing depth, the presence of bleeding on probing, and suppuration should be assessed regularly for the diagnosis of peri-implant diseases.\textsuperscript{75}

In this context, studies including the health status of the peri-implant tissue in their success criteria also assessed the presence or absence of suppuration from the peri-implant sulcus or pocket.\textsuperscript{17, 76} Histologic studies have shown an infiltration with large numbers of polymorphonuclear leukocytes in acutely named peri-implant soft tissues indicating the clinical diagnostic value of suppuration

**Radiographic Evaluation:**

Radiography is the most commonly used clinical tool to assess marginal bone level at implants and its changes over time. The technique of choice is the intra-oral radiographic technique. The reason is that this permits individual adjustment of the X-ray beam angulation relative to each individual fixture. In addition, the high resolution in intra-oral radiographs provides possibilities to evaluate the bone level. All radiographs of implants should be taken with the film/detector parallel to the implant and the X-ray beam directed perpendicular to it. Threaded implants have the advantage of making it easy to determine whether the implant has been depicted with correct vertical irradiation geometry or not. An intra-oral radiograph, however, only illustrates clearly the mesial and distal marginal bone levels and early bone loss often occurs on the facial aspect of the implant.\textsuperscript{77, 78} Marginal bone loss during the first year of loading has been reported to be at most 1-1.5 mm and thereafter less than 0.2 mm on an annual basis.\textsuperscript{38, 41, 79, 80} Little is known about the bone loss during the healing period. Astrand et al. (2004) started to radiographically monitor the marginal bone level at the time of implant insertion and found the bone loss between implant placement and prosthesis insertion to be several
times higher than between prosthesis insertion and a 5-year follow-up.\textsuperscript{81} Alternatively, more recent animal and clinical studies suggest that immediate functional loading of implants with sufficient primary stability may be considered a valid treatment alternative in single-tooth replacement.\textsuperscript{82} It has also been suggested that functional loading of implants may enhance osseointegration and does not result in marginal bone loss.\textsuperscript{83} However, it has been stated that radiographs are required to evaluate supporting bone levels around implants; and that cone beam radiography offers advantages in implant dentistry that osseous structures can be represented in three planes, true to scale and without overlay or distortion.\textsuperscript{75}

**Criteria of Implant Success: Changes over Time**

Over the years many researchers have proposed criteria for success regarding oral implants. One of the oldest, and most commonly used criterion was proposed by Albrektsson et al. (1986).\textsuperscript{50} Albrektsson proposed: Implant immobile when tested clinically; radiograph does not display evidence of peri-implant radiolucency; implant has absence of persistent and/or irreversible signs and symptoms such as pain, infections, neuropathies, paresthesia, or violation of the mandibular canal; vertical bone loss be less than 1 mm during the first year, and 0.2 mm annually following implant’s first year of service; and a successful rate of 85\% at the end of a five-year observation period and 80\% at the end of a ten-year period be a minimum criterion for success.\textsuperscript{50} This demand for marginal bone loss to be less than 0.2 mm annually after the first year of loading was met with much criticism for its rigidity. It has been pointed out that if the bone loss exceeds the yearly 0.2 mm, but then stabilizes and remains equal over a longer period the implant can still be considered clinically successful.\textsuperscript{84}
Other’s suggested success criteria that are similar to those by Albrektsson and co-workers (1986) with only minor differences. Albrektsson & Zarb (1993) suggested that each and every implant should be evaluated as a part of a four-grade scale representing success, survival, unaccounted for and failure. The success category includes implants that meet all of the success criteria according to Albrektsson et al. (1986), and the survival category are those attached implants that are not checked for mobility. The unaccounted for category includes all those patients who died or dropped out of the study, and the failure category includes all removed implants. Traditionally, implant survival was based upon the ability of the fixture to successfully osseointegrate within the alveolar bone. These requirements for successful osseointegration were introduced by Albrektsson in 1981, and included: biocompatibility, design, surface conditions, status of the host bed, surgical technique at insertion, and loading conditions applied afterwards. These requirements for successful osseointegration of the implant are however not necessarily interchangeable with clinical success. Secondary loss of osseointegration may be a frequent problem with respect to different biomaterials as well as implant designs.

Ongoing marginal bone loss is a factor affecting the outcome of implant treatment. If the marginal bone loss around an implant continues for several years, it may jeopardize the implant outcome. If the bone loss is recognized and treated, the implant might be saved. This topic has been evaluated with regards to implant position, size, and geometry of the implant, resulting in multiple factors with the potential of influencing the bone loss around implants. Bacterial colonization of the fixture-abutment
interface microgap was one of the factors reported in having an influence on this marginal bone position.\textsuperscript{18}

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<td>Schnitman &amp; Shulman (1979)</td>
<td>Bone loss no greater than a third of the vertical height of the implant</td>
<td>No suggested criteria</td>
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<td>Albrektsson et al. (1986)</td>
<td>Vertical bone loss &lt;0.2 mm annually following the implant’s first year in service</td>
<td>No evidence of peri-implant radiolucency</td>
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<td>Smith &amp; Zarb (1989)</td>
<td>Mean vertical bone loss &lt;0.2 mm annually after the first year in service</td>
<td>No evidence of peri-implant radiolucency as assessed on an undistorted radiograph</td>
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<td>Albrektsson &amp; Isidor (1993)</td>
<td>Average bone loss &lt;1.5 mm the first year in service, and thereafter &lt;0.2 mm annually</td>
<td>No evidence of peri-implant radiolucency</td>
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<td>Wennstrom &amp; Palmer (1999)</td>
<td>Maximum bone loss of 2 mm between prosthesis installation and the 5th year, with the majority of the loss occurring during the first year</td>
<td>No suggested criteria</td>
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<td>Ostman et al. (2007)</td>
<td>Success grade 1 &lt;2 mm bone loss the first year in service</td>
<td>No radiographic signs of pathology</td>
</tr>
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<td></td>
<td>Success grade 2 &lt;3 mm bone loss the first year in service</td>
<td>No radiographic signs of pathology</td>
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**Bacterial Colonization Patterns of Dental Implants and Peri-Implant Infections**

The soft tissue surrounding healthy osseointegrated dental implants share anatomical and functional features with the gingiva around teeth. The microstructure has been described in dog models and human tissues.\textsuperscript{90-92} The outer surface of the peri-implant mucosa is lined by a stratified keratinized oral epithelium continuous with a junctional epithelium attached to the titanium surface by a basal lamina
hemidesmosomes. The 2mm long non-keratinized junctional epithelium is in the apical portion only a few cell layers thick, separated from the alveolar bone by 1-2mm of collagen-rich connective tissue. This 3-4mm “biological barrier”, formed irrespective of the original mucosal thickness and protects the zone of osseointegration from factors released from plaque and the oral cavity.

Unlike the gingiva around teeth, the connective tissue compartment between the junctional epithelium and the alveolar bone consists of a scar like connective tissue almost devoid of vascular structures, greater amounts of collagen and fewer fibroblasts. However, more recently the same group examined a 40-μm-wide zone of connective tissue immediately lateral to the implant surface and found that it had many fibroblasts with a relatively low proportion of collagen. This may indicate that the fibroblast-rich barrier next to the titanium surface has a high cell turnover and that fibroblasts play an important role in establishing and maintaining the mucosal seal.

The inflammatory infiltrate in peri-implant tissue and the response to plaque accumulation have been described in animal models and humans. Similar to the disease process of gingivitis around natural teeth; an inflammatory infiltrate forms in the connective tissue as a response to the microbial colonization of the titanium surface. The infiltrate represents the local host-response to bacterial accumulation and proliferates in an apical direction when the time for plaque accumulation is prolonged. The peri-implant mucosa is similar to the gingiva around teeth as regards of function and immunology. An inflammatory cell infiltrate of equal size and composition has been found in clinically healthy tissues of gingiva and peri-implant mucosa. Immunohistochemical and immunological analysis show that the inflammatory infiltrate
consists of neutrophils, lymphocytes, macrophages and a few plasma cells. Intraepithelial antigen-presenting cells and adhesion molecules, such as ICAM-1 are expressed in epithelia adjacent to implants in a similar fashion as around teeth.103 The distribution of inflammatory cell phenotypes in healthy gingiva and peri-implant keratinized mucosa is also similar.104 Functional adaptation of the junctional epithelium occurs although its origin differs from that around the teeth.105

In dentate patients with implant sites adjacent to natural teeth, Furst et al. (2007) assessed subgingival plaque samples from implants and neighboring teeth with checkerboard DNA-DNA hybridization. Their results concluded that colonization of periodontal bacteria occurred within 30 minutes after the completion of trans-mucosal implant installation surgery. The authors also mentioned that the establishment of the microbiota was faster at tooth sites; and that different colonization patterns existed at tooth vs. implant sites, suggesting that transmission of pathogens is not immediately established.6

Bacterial colonization of edentulous patients has been studied in multiple investigations.3,106 Implantation of artificial fixtures in patients that are edentulous provides an interesting model for the investigation of shifts in the composition of the microbiota due to alterations of oral ecological conditions. In a study by Mombelli et al. (1988), emphasis was placed on a limited number of microorganisms known to be associated with various clinical oral conditions. Small amounts of bacteria were collected from the preoperative swabs. On an average 86% of the microorganisms were identified morphologically as coccoid cells and over 80% of the cultivated bacteria were Gram-positive facultative cocci. The authors concluded that after implant
installation, no significant changes in these proportions could be observed. Fusobacteria could only be detected in 13 of 104 samples. Black-pigmented *Bacteroides* were found infrequently and no trend of increase was apparent in any site over the 180 days of monitoring. Another study looking at microbiological features of implants placed in edentulous patients two years after implantation, was investigated by Mombelli et al. (1990). These results indicated that 52% of organisms were facultative anaerobic cocci and 17% were facultative anaerobic rods, while Gram-negative anaerobic rods accounted for only 7.3%.

In edentulous subjects *A. actinomycetemcomitans* and *P. gingivalis* are not as frequently associated with peri-implant infection as in dentate subjects. Danser et al. (1997) reported that after full-mouth extraction in patients with severe periodontitis, they could no longer detect the latter bacteria on the mucosal surface of edentulous patients, which shows that a shift in the microflora had occurred after total extraction. *A. actinomycetemcomitans* or *P. gingivalis* could not be isolated at the peri-implant pockets in these patients after insertion of implants.

In partly edentulous patients, the developing microbiota around implants closely resembles the microflora of naturally remaining teeth. A history of periodontitis, such as individuals susceptible to periodontal disease and the presence of putative periodontal pathogens are factors that can influence the maintenance and long-term prognosis of peri-implant tissues in the partly edentulous. Quirynen (1996) using phase contrast microscopy, examined partly edentulous subjects and evaluated the impact of periodontitis around remaining teeth and of probing depth around the implants on the composition of the peri-implant subgingival flora. They found that the
subgingival microflora around implants harbored more spirochetes and motile rods when there were teeth present in the same jaw. The patients were deemed healthy, or as having chronic or refractory periodontitis. Samples from deep peri-implant pockets (≥4mm) in patients with chronic or refractory periodontitis showed significantly higher proportions of spirochetes and motile rods than those with comparable probing pocket depth in periodontally healthy patients.

Papaioannou et al. (1996) using phase contrast microscopy and DNA probes, determined the prevalence of putative periodontal pathogens in partly edentulous and edentulous patients with a history of periodontal disease. Their microbiological profiles were similar around teeth and dental implants of equal pocket depth, which confirmed the hypothesis that pockets around teeth can act as a reservoir for putative periodontal pathogens. This finding has been confirmed by several clinical studies of partly edentulous patients. As early as one month after implantation, putative periodontal pathogens can be detected around the implants of partly edentulous patients.

Implant failures due to infection are characterized by a complex peri-implant microbiota resembling that of adult periodontitis. Apart from dark-pigmented Gram-negative anaerobic rods, other bacterial species that associated with peri-implant infection include B.forsythus, F.nucleatum, Campylobacter, P.micros and S.intermedius. Other organisms not primarily associated with periodontitis, such as Staphylococcus spp, enterics and Candida spp have also been found in peri-implant infections. The longitudinal data available on the microbial colonization of implants in partly edentulous persons with a history of periodontal disease have shown no
association between periodontal pathogens around teeth and implants with loss of attachment during 36 months function of implants.\textsuperscript{112,117}

Differences in the microbiota for healthy sites as well as peri-implantitis has also been investigated.\textsuperscript{107,116,118} In 1987 Mombelli et al. found that sites associated with failing implants were characterized by a complex microbiota with a large proportion of Gram-negative anaerobic rods. Black-pigmented \textit{Bacteroides} and \textit{Fusobacterium} spp. were regularly found. Spirochetes, fusiform bacteria as well as motile and curved rods were a common feature in the darkfield microscopic specimens of these sites. Healthy sites in the same patients harbored small amounts of bacteria. The predominant morphotype was coccoid cells. Spirochetes were not present, fusiform bacteria, motile and curved rods were found infrequently and in low numbers.\textsuperscript{107} Another study, further supporting the differences in microbiota between healthy and diseased peri-implant tissues, was investigated by Leonhardt et al. in (1999). The two types of clinical conditions showed distinct bacterial profiles. For implants with peri-implantitis, putative periodontal pathogens, such as \textit{Porphyromonas gingivalis}, \textit{Prevotella intermedia}, \textit{Prevotella nigrescens} and \textit{Actinobacillus actinomycetemcomitans}, were found in 60\% of the cases and microorganisms primarily not associated with periodontitis, such as \textit{Staphylococcus} spp., enterics and \textit{Candida} spp., were found in 55\% of the peri-implant lesions. In contrast, implants surrounded by healthy tissue demonstrated a microbiota associated with periodontal health. The results indicate that the microbiota of the healthy peri-implant sulci is similar to that from corresponding conditions around teeth. However, in peri-implant areas staphylococci, enterics and yeasts were found
almost as frequently as periopathogens indicating differences as compared to the microbiota around periodontitis affected teeth.\textsuperscript{116}

To ensure maintenance and long-term stability of osseointegrated dental implants, it is essential to study the relation between microbial provocation and the inflammatory reaction. The inflammation caused by the microbiota probably varies between subjects, as shown in patients with different types of periodontal disease. Individuals positive for the gene encoding for interleukin-1β (allele 2 of IL-1β at +3953) produce up to four times more IL-1β.\textsuperscript{119} Patients with failing implants have been shown to have a “hyper-inflammatory trait” unlike those with only successful and clinically healthy implants.\textsuperscript{120} Therefore, the same bacterial stimuli may cause greater tissue destruction in persons with an aberrant host response.

Peri-implantitis is defined as an inflammatory reaction with loss of supporting bone in the tissues surrounding a functioning implant.\textsuperscript{121} It is also been described as “a site specific-infection yielding many features in common with chronic adult periodontitis” or “an inflammatory, bacterial-driven destruction of the implant-supporting apparatus.”\textsuperscript{107,122} A cause-related effect between plaque accumulation and peri-implant mucositis has been shown in animals and humans.\textsuperscript{98,99} Moreover the microbial colonization of implants follows the same pattern as around teeth.\textsuperscript{112} During peri-implant breakdown a complex microbiota is established closely resembling that found in adult periodontitis.\textsuperscript{107,113,114} Implants displaying deeper probing depths associated with bone loss and bleeding on probing have been found to harbor \textit{A. actinomycetemcomitans} and \textit{P. gingivalis}.\textsuperscript{114,123} It was partly due to these findings and the virulence factors associated with their pathogenesis that these bacteria were chosen for our investigation.
**P. Gingivalis**

*Porphyromonas gingivalis* is a Gram-negative, non-motile, rod-shaped, anaerobic organism. To function, it undergoes a mechanism in which it binds to and inhabits the subgingival architecture of the mouth using fimbriae. *P. gingivalis* is considered an important member of the microbiota involved in periodontal disease progression and bone and tissue destruction. This organism is present in very low levels during periodontal health, while during the disease progression of periodontitis can reach significant numbers. Putative periodontal pathogens such as *P. gingivalis* have been found colonizing dental implants within 30 minutes of surgical installation. Leonhardt et al. (1999) provided evidence of implants with peri-implantitis, associated with periodontal pathogens, such as *Porphyromonas gingivalis*. *P. gingivalis* has the unique ability to invade epithelial cells and therefore providing a mechanism by which to escape the protective innate immune response. *P. gingivalis* also produces many cell components and macromolecules that function as virulence factors. Noted virulence factors include the lipopolysaccharide, proteinases, and collagenases. This bacterium is a highly pathogenic and virulent member of the subgingival plaque biofilm.

**A. Actinomycetemcomitans**

*Aggregatibacter actinomycetemcomitans* is a facultative gram-negative bacterium, which has been associated with severe oral and non-oral infections. *A. actinomycetemcomitans* has also been associated with peri-implant diseases as evidenced by Leonhardt et al. (1999) and has also been connected with breakdown resembling that of periodontitis lesions around implants (peri-implantitis). *A. actinomycetemcomitans* has been shown to possess a variety of virulence factors that not only enhance its survival in the oral cavity but also contribute to the pathogenesis of
periodontitis.\textsuperscript{127} \textit{A. actinomycetemcomitans}' ability to attach to extracellular matrix proteins and epithelial cells is due to the benefit of adhesins and invasins.\textsuperscript{128} Once the organism is established in the oral tissues, the host reacts to the bacterial insult with an inflammatory response resulting in the destruction of periodontal tissues in a susceptible host.\textsuperscript{129} \textit{A. actinomycetemcomitans} contains a number of factors causing bone resorption including lipopolysaccharide, proteolysis-sensitive factor, and GroEL.\textsuperscript{127} These factors are compounded with its other effects on the connective tissue and extracellular matrix such as collagenase and fibroblast cytotoxin.\textsuperscript{128}

**Dental Implant Design and Abutment/Fixture Geometry**

Although the basic form of the endosseous dental implant has remained relatively unchanged, the material composition, surface modifications, and abutment connection designs have since changed dramatically. There are two basic concepts in implant design; implants that are manufactured to be placed at the tissue level, or at the bone level. The rational behind a tissue level implant is that this concept raises the fixture abutment interface to a supragingival level possibly alleviating the effects of a microgap between the fixture and abutment, however this design may have esthetic implications by not allowing for optimal restorative contours or emergence profile. The bone level implant design allows for a more apical connection of the fixture abutment interface. This apical connection provides a more ideal emergence profile for the restoration, creating a more natural and esthetic prosthesis. Bone level implant designs have however been shown to be associated with crestal bone loss after installation.\textsuperscript{130, 131}

The original implants designed by Brånemark were created with commercially pure titanium using an external hex abutment connection and incorporated a machined external surface. The external hex refers to the implant abutment interface, where the
implant displays a hexagonal connection for which the abutment will be seated (Figure 2-7). This design originally was present to help screw the implant into place. The external hex design allows for an abutment connection outside of the implant. This connection not only provided indexing of single unit abutments but also enabled multiple implants to be rigidly splinted together via a metal bar for a fixed prosthesis and allow passive connection to the implants. To date this implant has supporting documentation spanning over three decades.²⁶, ³⁰, ¹³² Although this implant was the premier fixture of its time, structural quality and patient’s demands have created a paradigm shift in implant design, where recent attention has focused on the fixture surface and the implant abutment interface. The external hex has been replaced in many fixtures with an internal hex design. This internal connection refers to the hexagonal interface now being inside the implant where the abutment is secured. This connection was designed to provide a more ridged connection, enhance implant strength, improve the seal of the fixture and abutment interface, and to medialize this connection.

Figure 2-7. Implants displayed light with dark abutments A) External hex implant abutment connection with screw fixation, B) Internal Morse-taper connection with threaded solid abutment fixation, C) Internal implant abutment connection with solid threaded abutment fixation, D) Internal Morse-taper connection with a press fit fixation.
Another concept that must be realized in understanding implant design is one versus two-stage implant surgery. One stage implant surgery refers to placement of a healing abutment following implant installation that remains transmucosal and exposed to the oral cavity following replacement of the mucoperiosteal flaps. In contrast, during two-stage implant surgery a cover screw is placed following implant installation and the implant is completely submerged following suturing of the flaps. Three to six months later the implant is uncovered with a second surgical procedure and a healing abutment is placed allowing the peri-implant mucosa to heal. Although the one-stage technique has less morbidity for the patient, since it involves a single surgical procedure, the two-stage surgery might offer greater potential for soft tissue management.

This concept of platform switching was developed to control bone loss after implant placement and refers to the use of an abutment of smaller diameter connected to an implant neck of larger diameter.\(^8^9\) A “platform switched” connection shifts the perimeter of the implant-abutment junction inwards towards the middle of the implant improving the distribution of forces while medializing this junction. The inward movement of the implant abutment interface is believed to shift the inflammatory cell infiltrate to the central axis of the implant and away from the adjacent crestal bone.\(^8^9\)

Degidi et al. 2007, explained that when the horizontal relationship between the outer edge of the implant and a smaller-diameter component (“platform switching”) is altered, a reduction to crestal bone loss occurs.\(^1^3^3\) Other authors have supported these findings as well, proving evidence for decreased crestal bone loss following a platform switching concept.\(^1^3^4-1^3^6\) The ability to reduce or eliminate crestal bone loss would be a major achievement in implant dentistry.
In a two-piece implant system the abutment is retained in the fixture using mechanical attachment. This attachment can sometimes result in gaps between the implant and abutment resulting in a bacterial reservoir.\textsuperscript{10} Bacteria in this connection gap may cause an inflammatory process in the peri-implant tissues leading to peri-implant disease and possible marginal bone loss. The introduction of the internal hex implant gave way for the Morse tapered connection. This internal Morse taper design that some manufactures have introduced, essentially seals the abutment to the fixture with this tapered connection. The principle of the Morse-taper is that of a cone within a cone, where the male portion and the female portion are both uniformly tapered creating intimate contact. Implant taper compresses the walls of the abutment as it expands. Thus, the stresses inside the materials keep both components fixed together. Intimate metal-to-metal contact of a true Morse-taper, may prevent micromovement and transfer the biological width area to a horizontal rather than vertical direction allowing for hard tissue formation above the fixture-abutment interface.\textsuperscript{137} Different internal connections
have been evaluated in a recent study where the Morse tapered designs were the only implants failing to show any movement between the abutment and fixture. The results of this study suggested this lack of movement might prevent the pumping of bacterial contaminated liquid from in and out of the implant, resulting in a decrease of marginal bone loss. The evidence of this Morse tapered design is depicted in our test group.

**Abutment/Fixture Junction Geometry: Laboratory Studies**

In vitro studies evaluating microbial penetration along the internal part of dental implants have been reported utilizing implants with different fixture-abutment interface geometries under loading and non-loading conditions. In addition, there are laboratory tests utilizing finite element analysis trying to demonstrate and evaluate stress patterns on peri-implant bone as well as studies looking into the movement of different fixture-abutment components.

Under non-loading conditions, Quirynen et al. (1994) demonstrated that when fixtures with an external hex design and abutments were assembled and installed in a liquid blood medium inoculated with oral microorganisms, bacterial invasion of the fixture-abutment interface microgap was detected. Similarly, Jansen et al. (1997) reported microbial leakage of thirteen different implant-abutment combinations using *E.coli* as indicator bacteria. Among the different implant-abutment combinations an implant with an internal connection and a silicon washer demonstrated the fewest cases of leakage. Aloise et al. (2010) compared the frequency of bacterial leakage of *Streptococcus sanguinis* along the implant-abutment interface between two systems of Morse taper dental implants under non-loading conditions. Different methods of activation of the taper abutments were used: tapped-in and screwed-in. Irrespective of which of the two Morse taper implant connection systems of activation was analyzed,
this in vitro experiment showed bacterial leakage along the implant-abutment interface.\textsuperscript{139}

There is limited information from in vitro studies evaluating microbial contamination of the fixture-abutment interface microgap under loading conditions. A study by Steinebrunner et al. (2005) evaluated microbial leakage between implants and their abutments using a loading protocol. However, although bacterial leakage along the interface was shown for all tested implants, the number of load cycles until bacterial penetration occurred differed significantly between implant systems and their connection designs. Specifically, implants with a tri-channel internal connection showed bacterial leakage at significantly higher numbers of chewing cycles compared to implants with external hex, implants with internal connection and a silicon washer, and implants with internal hex with friction fit connection.\textsuperscript{16} Koutouzis et al. (2010) utilized an in vitro dynamic loading model to assess the potential risk for invasion of oral microorganisms into the fixture-abutment interface microgap of dental implants with different fixture-abutment connection characteristics. In this experiment twenty-eight implants were divided into two groups (n=14/group) based on their microgap dynamics. Group 1 was comprised of fixtures with internal Morse-taper connection that connected to standard abutments. Group 2 was comprised of implants with a four-groove conical internal connection that connected to multi-base abutments. The specimens were immersed in a bacterial solution of \textit{Escherichia coli} and loaded with 500,000 cycles of 15 N in a wear simulator. Following disconnection of fixtures and abutments, microbial samples were taken from the threaded portion of the abutment, plated and cultured under appropriate conditions. The difference between loosening and tightening torque
value was also measured. One of the 14 samples in Group 1 and 12/14 of samples in Group 2 developed multiple colony forming units (CFU) for \textit{E.coli}. Implants in Group 1 exhibited an increase in torque value in contrast to implants in Group 2 that exhibited a decrease. This study indicated that differences in implant design may affect the potential risk for invasion of oral micro-organisms into the fixture-abutment interface microgap under dynamic loading conditions.

Maeda et al. utilized a 3D finite element model to examine the biomechanical advantages of platform switching. He notes that this procedure shifts the stress concentration away from the bone-implant interface, but these forces are then increased in the abutment or the abutment screw.\textsuperscript{140}

Crestal bone changes around implants are significantly influenced by micromovements between the abutment and the implant as shown in animal studies.\textsuperscript{13} Micromovements of the fixture-abutment interface have been evaluated recently with an in \textit{vitro} study by Zipprich et al. (2007). Implants from different manufacturers with their respective abutments were loaded up to 200N at an angle of 30 degrees and filmed during force application with a high-speed camera. Micromovement, creating microgaps were recorded in all but 2 of the 9 implant systems, one of which was the Morse-taper implant described in our study.\textsuperscript{138}

Thus, it can be deduced that an implant with an internal Morse-taper connection, devoid of a microgap created by micromovement between the abutment and the implant, may minimize the effect of bacterial infiltrate on the peri-implant tissues.
Abument/Fixture Junction Geometry and the Effects of Placement Position on the Peri-Implant Tissues: Animal Studies

The location of the fixture-abutment interface can be placed in various positions in relation to the alveolar crest (crestal, supracrestal, subcrestal). The location of the fixture-abutment interface can be of major importance when the creating esthetic restorations. Placement of the fixture-abutment interface in a more apical position can create an ideal emergence profile for the prosthetic construction.141

Subcrestal position of the fixture-abutment interface has been reported to have a negative influence on marginal bone level changes in a few animal studies.130, 131, 142, 143 In an experimental study in dogs, Hermann et al. (2000) reported that placement of two-part implants with the fixture-abutment interface 1 mm below the crestal bone resulted in pronounced crestal bone loss following 6 months of healing. In this study the authors used custom-made implants with a fixture-abutment interface micro-gap of 50 μm.130 Similarly, Jung et al. (2008) evaluated the influence of non-matching implant and abutment diameters on radiographic crestal bone levels in dogs. Radiographic analysis revealed very little bone loss and a slight increase in bone level for implants placed at the level of the crest or 1 mm above. The greatest bone loss occurred at implants placed 1 mm below the bone crest. No clinically significant differences regarding marginal bone loss and the level of the bone-to-implant contact were detected between implants with a submucosal or a transmucosal healing. However, the amount of crestal bone loss was smaller compared to that found in the study by Hermann et al. (2000).131

In a similar animal experiment, Todescan et al. (2002) evaluated the healing around implants (Brånemark System) that were placed either 1 mm above, level with or 1 mm below the crestal bone. Here it was reported that the first marginal bone to
implant contact was located between 1.6 mm and 2.5 mm apical to the fixture-abutment interface with the shortest implant contact distance associated with implants that were placed in the subcrestal position. Similar findings have been reported by Pontes et al. (2008) where they placed implants with the fixture-abutment interface at the bone crest, 1 mm and 2 mm apical to this position. Following 4 months of healing all implant groups had the first bone to implant contact apical to the fixture-abutment interface. None of these animal studies reported bone formation above the fixture-abutment interface when implants are placed in a subcrestal position. In contrast to the previously described studies, few animal experiments have reported favorable outcomes for implants in a subcrestal position with bone formation close to or even above the fixture-abutment interface. Welander et al. (2009) observed osseointegration coronal to the fixture-abutment interface when placing implants with the fixture-abutment interface 2 mm subcrestally. The test implants in this study had a surface modification extending to the implant margin that included the shoulder part of the implant and a conical interface between the abutment and the implant. Similar findings were reported by Weng et al. (2008), showing that implants with subcrestal position presented bone growth onto the implant shoulder in nearly all histological sections. Implants utilized in this study contained a reduced abutment diameter in relation to the fixture diameter, a Morse-taper implant-abutment connection, and a microstructured surface treatment which included the cervical collar and extended onto the implant shoulder.

Understanding implant placement at different bone level heights and its effects on marginal bone loss becomes imperative when selecting an implant design. Even though one-stage transmucosal implants exhibit stable peri-implant bone levels when
the fixture abutment interface is located supracrestal and the border between rough and smooth surface is located at the alveolar crest it seems that placement of the border between the rough and smooth surface below the bone crest can lead to marginal bone loss and it is not recommended. When placing implants at a subcrestal position, an internal connection with a reduced diameter abutment and a Morse-taper design may have positive effects on marginal bone levels compared to other designs.

Abutment/Fixture Junction Geometry: Human Studies

The effects of the fixture-abutment interface have been evaluated in few clinical studies.\textsuperscript{89, 133, 146-148} The number of studies that benefit from histology are further reduced.\textsuperscript{133, 146} Histologic and radiographic observations suggest however that a biologic dimension of hard and soft tissues exists around dental implants and extends apically from the implant-abutment interface. This clinical evidence however is beneficial especially when focusing on the comparisons of differing fixture-abutment interfaces in relation to marginal bone loss.\textsuperscript{89}

In vivo studies evaluating histology of peri-implant tissues have been reported in the literature.\textsuperscript{133, 146} Romanos et al. (2005) evaluated biopsies from human implants. This histologic and histomorphometric analysis on the interface of immediately loaded implants retrieved showed a high percentage of bone-to-implant contacts after a loading period of 2 and 10 months. This observation was independent of the implant system and fixture-abutment interface used. The examined implants had a screw-geometry and rough surfaces to promote new bone formation at the initial stages of healing during loading.\textsuperscript{146} Degidi et al. (2008) explained that when the horizontal relationship between the outer edge of the implant and a smaller-diameter component ("platform switching") is altered, a reduction to crestal bone loss occurs. After histomorphometric analysis of
three Morse-taper connection implants the author concludes that when there is zero microgap and no micromovement, platform switching shows no resorption and better esthetics.\textsuperscript{133}

Lazzara et al. (2006) retrospectively discovered that matching-diameter prosthetic components were not available, and many of the early 5.0- and 6.0-mm-wide implants received "standard"-diameter (4.1-mm) healing abutments and were restored with "standard"-diameter (4.1-mm) prosthetic components. Long-term radiographic follow-up of these "platform-switched" restored wide-diameter dental implants has demonstrated a smaller than expected vertical change in the crestal bone height around these implants than is typically observed around implants restored conventionally with prosthetic components of matching diameters. This radiographic observation suggests that the resulting post-restorative biologic process resulting in the loss of crestal bone height is altered when the outer edge of the implant-abutment interface is horizontally repositioned inwardly and away from the outer edge of the implant platform. This article introduces the concept of platform switching and provides a foundation for future development of the biologic understanding of the observed radiographic findings.\textsuperscript{89}

Current clinical studies utilizing two-piece implant systems with an altered horizontal relationship between the fixture diameter and the abutment diameter, report minimal marginal peri-implant bone loss.\textsuperscript{147-149} In a 5-year prospective study Wennström et al. (2005) reported mean bone level changes from the time of crown placement to the first year follow up of 0.02 mm measured on implant level.\textsuperscript{148} Norton et al. (2006) reported an average of marginal bone loss of 0.65 mm from implant therapy in 54 patients where the implants had been in function for 37 months.\textsuperscript{149}
Taken together, the results of in-vitro studies show that differences in implant design may affect the potential risk for invasion of oral micro-organisms into the fixture-abutment interface under non-loading and dynamic loading conditions. Implants with internal Morse-taper connection have the highest potential to prevent bacterial contamination of the fixture-abutment interface. The results from animal studies demonstrate that implants with reduced abutment diameter in relation to the fixture diameter, a Morse-taper implant-abutment connection and a microstructured surface treatment which included the cervical collar and extended onto the implant shoulder can maintain stable peri-implant bone levels even when the fixture-abutment interface is placed in a subcrestal position. These results are in line with clinical studies showing that implants with reduced abutment diameter in relation to the fixture diameter and a Morse-taper implant-abutment connection exhibit less marginal bone loss compared to implants with an external hex connection at least at the earlier stages of healing.

With these conclusions some of which were reported after the experimental conclusion of my project, is was the aim of my study to use an in vitro model to assess the potential risk for invasion of oral microorganisms into the fixture-abutment interface microgap in dental implants with different internal connection designs. With this knowledge it is possible to reinforce previous conclusions that differences in implant design may affect the potential risk for colonization of oral microorganisms into the fixture-abutment interface microgap, which may ultimately influence the peri-implant tissues eg. marginal bone
CHAPTER 3
MATERIALS AND METHODS

Implant Experiment Groups

For this study, three groups of implants were compared based on their FAI microgap geometry. Ten implants were tested in each experimental group: group 1: fixtures with an internal Morse-taper connection were connected to standard straight abutments with a height of 6-mm (Fig. 3-1); the abutments were connected to the fixtures with a torque of 25 Ncm using the appropriate torque wrench according to the manufacturer’s protocol; group 2: identical fixtures and abutments as described in group 1 were used with the exception that prior to fixture–abutment connection, a vertical groove of 0.5-mm depth was prepared with a fissure bur on one side of the abutment (Fig. 3-2). The fixtures and abutments were connected using a torque of 25 Ncm using the appropriate torque wrench according to the manufacturer’s protocol. The introduction of a 0.5-mm groove to the abutment was to ensure microbial penetration to the internal part of the implant, while allowing for the exact same torque for connecting the abutment as the implants in group 1; group 3: fixtures with a tri-channel internal connection were connected to 3-mm high abutments. The components were connected with a torque of 35 Ncm using the appropriate torque wrench according to the manufacturer’s recommendation.

To evaluate the microbial detection techniques, two standard straight abutments with a height of 6-mm were used. These abutments correlated to one negative and one positive control abutment. The negative-control abutment was not connected to a fixture and was not subjected to bacterial culture to ensure an uncontaminated laboratory environment and avoid false positive results. The positive-control abutment
was not connected to a fixture but was subjected to the same multi species bacterial culture containing *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* as groups 1 through 3, in order to assure cultivatable bacteria were in fact present in the broth and that our swabbing and culturing techniques were reliable.

All fixtures and abutments were connected in a sterile environment and placed in a plastic container with the multi species bacterial solution covering the FAI interface and containing microorganisms as described below.

**Bacterial Culture Conditions**

*Aggregatibacter actinomycetemcomitans* VT1169 (State University of New York [SUNY] 465 nalidixic acid resistant rifampicin resistant) was grown in liquid tryptic soy broth supplemented with yeast extract and cultured at 37°C in 10% CO2 to the mid-logarithmic phase. *Porphyromonas gingivalis* W83 was grown in liquid tryptic soy broth supplemented with hemin, vitamin K, yeast extract, and L-cysteine hydrochloride at 37°C under anaerobic conditions to the mid-logarithmic phase. Implants were placed in an aliquot of a 1:10 dilution of a 1:1 stock solution of A. actinomycetemcomitans VT1169 and P. *gingivalis* W83 multi species broth and incubated at 37°C under anaerobic conditions for 5 days.

**Microbial Sampling and Detection**

After disconnection of fixtures and abutments under sterile conditions, microbial samples were taken from the threaded portion of the abutment using sterile calcium alginate swabs. Samples were plated with the calcium alginate swabs directly onto tryptic soy broth agar plates supplemented with yeast extract for the detection of *A. actinomycetemcomitans* and onto tryptic soy-broth agar plates supplemented with hemin, vitamin K, yeast extract, and L-cysteine hydrochloride for detection of P.
gingivalis colony forming units (CFUs). Plates A. actinomycetemcomitans and P. gingivalis for were incubated at 37°C in 10% CO2 for 2 and 7 days, respectively. Individual CFUs were counted and recorded.

**Statistical Analyses**

Median values and interquartile ranges were calculated for the number of CFUs for A. actinomycetemcomitans and P. gingivalis. In addition, the total number of implants per group exhibiting bacterial colonization of the FAI microgap was calculated. The Kruskal-Wallis test with Dunn’s multiple comparisons was applied to evaluate differences among the three groups regarding the number of CFUs for A. actinomycetemcomitans and P. gingivalis. The X² test was used to evaluate differences in the number of implants exhibiting bacterial colonization of the FAI microgap among the different groups. A P value <0.05 was considered significant.

Figure 3-1. A) Implant of group 1, B) Abutment of group 1, C) Implant of group 3, D) Abutment of group 3.
Figure 3-2. Standard straight abutment of group 2 with 0.5 mm vertical groove.
Figure 3-3. Implant and abutment of group 1 and group 3 (left) in a plastic container with the bacterial solution.
CHAPTER 4
RESULTS

To validate the colonization and detection techniques, abutments similar to those in group 1 and group 3 were left unassembled and either exposed to bacterial culture or left sterile acting as positive and negative controls. Zero CFUs of A. actinomycetemcomitans or P. gingivalis were detected from sampling of abutments that were left sterile (negative control), whereas 188 CFUs of A. actinomycetemcomitans and 113 CFUs of P. gingivalis were detected in samples from abutments exposed to bacterial culture (positive control). These data indicate that the conditions for colonization and sample collection were appropriate for the experimental design.

To semi-quantitate the ability of A. actinomycetemcomitans or P. gingivalis to colonize the fixture abutment interface microgap, CFUs from cultured samplings were quantified (Table 4-1). Group 1 exhibited significantly lower numbers of CFUs for A. actinomycetemcomitans (median: 0; interquartile range: 0 to 1) compared to group 2 (median: 81; interquartile range: 44.5 to 96.5) (difference: -36.25; P <0.05) and group 3 (median: 24.5; interquartile range: 11 to 56.5) (difference: -22; P <0.05). There was a significant difference in the number of CFUs for P. gingivalis between group 1 (median: 0; interquartile range: 0 to 0) and group 2 (median: 55; interquartile range: 35.5 to 96) (difference: -35.8; P <0.05). However, the difference in the number of CFUs for P. gingivalis between group 1 and group 3 (median: 12, interquartile range: 6 to 29.5) did not reach a statistically significant level (difference: -19.05; P >0.05).

The number of implants that had an FAI microgap contaminated with A. actinomycetemcomitans and P. gingivalis according to the different implant groups is presented in Table 4-2. Three of ten implants of group 1 had FAI microgaps colonized
by A. actinomycetemcomitans, whereas none of the implants of this group had FAI microgaps colonized by P. gingivalis. In contrast, 10 of 10 implants in group 2 and nine of 10 implants in group 3 had FAI microgaps colonized by both A. actinomycetemcomitans and P. gingivalis. There was a statistically significant difference for the number of implants that had FAI microgaps colonized by A. actinomycetemcomitans between groups 1 and 2 ($x^2 = 10.76; P <0.05$) and between groups 1 and 3 ($x^2 = 7.5; P <0.05$). Similarly, there was a statistically significant difference between groups 1 and 2 ($x^2 = 20; P <0.05$) and between groups 1 and 3 ($x^2 = 16.36; P <0.05$) regarding the number of implants that had FAI microgaps colonized by P. gingivalis.

Table 4.1. Median number of colony forming units (interquartile range) for A. actinomycetemcomitans and P. gingivalis by implant group

<table>
<thead>
<tr>
<th>Group</th>
<th>A. actinomycetemcomitans (CFU)</th>
<th>P. gingivalis (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=10)</td>
<td>0 (0 to 1) *†</td>
<td>0 (0 to 0) ‡</td>
</tr>
<tr>
<td>2 (n=10)</td>
<td>81 (44.5 to 96.5)</td>
<td>55 (35.5 to 96)</td>
</tr>
<tr>
<td>3 (n=10)</td>
<td>24.5 (11 to 56.5)</td>
<td>12 (6 to 29.5)</td>
</tr>
</tbody>
</table>

* P <0.05; group 1 versus group 2 for A. actinomycetemcomitans (Kruskal-Wallis test with Dunn comparisons).
† P <0.05; group 1 versus group 3 for A. actinomycetemcomitans (Kruskal-Wallis test with Dunn comparisons).
‡ P <0.05; group 1 versus group 2 for P. gingivalis (Kruskal-Wallis test with Dunn comparisons).
§ P <0.05; group 1 versus group 3 for P. gingivalis (Kruskal-Wallis test with Dunn comparisons).

Table 4.2. Number of implants with a fixture abutment interface microgap contaminated with A. actinomycetemcomitans and P. gingivalis by implant group

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Implants Contaminated With A. actinomycetemcomitans</th>
<th>Number of Implants Contaminated With P. gingivalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=10)</td>
<td>3 *†</td>
<td>0 ‡§</td>
</tr>
<tr>
<td>2 (n=10)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>3 (n=10)</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

* P <0.05; group 1 versus group 2 for A. actinomycetemcomitans ($x^2$ test).
† P <0.05; group 1 versus group 3 for A. actinomycetemcomitans ($x^2$ test).
‡ P <0.05; group 1 versus group 2 for P. gingivalis ($x^2$ test).
§ P <0.05; group 1 versus group 3 for P. gingivalis ($x^2$ test).
The present study shows that the tested dental implants with a Morse taper internal connection had negligible bacterial penetration down to the threaded part of the FAI under in vitro conditions compared to that of a tri-channel internal connection. The Morse taper is a method used by machinists to reliably join two rotating machine components. The principle of the Morse taper is that of the cone in the cone. The trunnion (the male portion) and the bore (the female portion) are both uniformly tapered. When the trunnion of the abutment is tapped or screwed into the bore of the implant fixture they come into intimate contact. The conical taper compresses the walls in the bore as it expands. Thus, the stresses inside the materials keep both components fixed together.\textsuperscript{151} The orthopedic industry has adapted these tapers, under the generic name of Morse tapers, as a means of reliably joining modular components of total joints directly on the operation table.\textsuperscript{152}

Figure 5-1. Internal Morse taper connection with threaded solid abutment fixation.\textsuperscript{153}
The Morse taper lock guarantees a superior mechanical stability compared to the external hexagonal connections, or butt joint design. This results in a better short and long term clinical performance. In a recent 4-year prospective clinical study on 1,920 Morse taper connection implants used in different prosthetic applications, high survival (97.5%) and success rates (96.6%) were reported. A mean distance from bone crest to implant shoulder of 1.07 mm and very few prosthetic complications at the implant abutment interface (0.65%) was found. In an 8-year study on 275 single tooth restorations with Morse taper connection implants, Doring et al. (2004) reported an implant survival rate of 98.2%, with no mechanical complications associated with the prosthetic components at the implant abutment interface. In another similar study on single tooth Morse taper connection implants with a mean follow-up period of 6.3 years, Weigl found a very low percentage (1.3%) of abutment loosening. These results were confirmed by a recent study on 307 Morse taper connection implants, with a four year follow-up, where high survival (98.4%) and success rates (97.07%) were reported, with a mean distance from bone crest to implant shoulder of 1.14 mm and a very low incidence of mechanical complications (0.66% abutment loosening). The results of these studies are in accordance with previous work on Morse taper connection implants, in which the use of tapered abutment connection, providing high resistance to bending and rotational forces during clinical function, reduced the risk of abutment loosening at the implant abutment interface.

Features of the implant abutment connection were considered to influence not only the mechanical behavior, but also the biologic behavior of implants. Stability of the implant abutment connection has been addressed to eliminate screw loosening, but
also to distribute load more favorably in bone \textsuperscript{154, 155, 160}. The effect of implant abutment design on marginal bone level is, however, highly debatable \textsuperscript{160, 161}. Some authors have suggested that micro-movements at the implant abutment interface could lead to bone resorption \textsuperscript{162, 163}. This hypothesis still has to be tested, but Morse taper connection implants can certainly avoid micro-movements at the implant abutment interface, preventing crestal bone loss around implants \textsuperscript{14}. Marginal bone stability has always been considered one of the most important reference criteria to evaluate implant success over time \textsuperscript{50}.

Some authors have advocated that a higher bacterial contamination may be related to a misfit at the implant abutment interface caused by screw loosening \textsuperscript{164, 165}. Screw loosening can damage interfaces in implant components, favoring contamination of their internal parts by microorganisms. Bacterial leakage between implants and abutments occurs and this leakage is higher when the abutment screw is tightened and loosened repeatedly \textsuperscript{164, 165}. For these reasons, the Morse taper implant abutment connection could provide an efficient seal against microbial penetration, significantly reducing the microgap dimensions at the implant abutment interface, and contributing to a minimal level of peri-implant tissue inflammation \textsuperscript{166}. With Morse taper connection implants, the gap is closed so tightly that the abutment and the fixture behave like a single piece. For this reason, there is effectively no microgap and no bacterial leakage \textsuperscript{166}. With the tapered interference, the abutment emergence geometry leads to “platform-switching” advantages \textsuperscript{89, 167}. Lazzara and Porter were the first authors to discover that the placement of platform-switched implants resulted in a smaller vertical change in the crestal bone level than was typically seen when restoring conventional
implants with abutments of matching diameter.\textsuperscript{89} The biologic rationale of the platform switching design or horizontal offset at the implant abutment interface is actually explained as the consequence of the horizontal repositioning of the microgap.\textsuperscript{167, 168} Basically, the principle involved is to distance the abutment fixture microgap away from the bone as far as possible. This is very important, because the microgap harbors bacteria that produce toxins; if bacteria are more distant from the bone, it is subsequently possible to minimize bone loss.\textsuperscript{89, 166-170}

Three of our 10 implants with this Morse taper connection (group 1) had one CFU of \textit{A. actinomycetemcomitans}. In addition, none of those implants developed CFUs for \textit{P. gingivalis}. These results seem to be relevant with the geometry of the internal connection; because nine of 10 implants with a tri-channel internal connection (group 3) developed multiple CFUs for both \textit{A. actinomycetemcomitans} and \textit{P. gingivalis}. However, there was no statistically significant difference between implants of groups 1 and 3 regarding the number of CFUs of \textit{P. gingivalis}.

Microbial penetration along the internal part of dental implants was reported in some in vitro studies using implants with different geometries of the FAI.\textsuperscript{8, 16, 18} For instance, Quirynen et al. (1994) demonstrated that bacterial invasion of the FAI microgap was detected when fixtures and abutments were assembled and installed in a liquid blood medium inoculated with oral microorganisms.\textsuperscript{8} Similarly, Jansen et al. (1997) reported microbial leakage of 13 different implant abutment combinations using \textit{E. coli} as the indicator bacteria.\textsuperscript{18} In addition, an in vivo study by Quirynen and van Steenberghe (1993) reported the presence of microorganisms in the inner threads of external hex implants.\textsuperscript{7} All screw threads in this study harbored significant quantities of
microorganisms. Most recently, Callan et al. (2005) described moderate to high levels of eight different periodontopathogenic microorganisms, including A. actinomycetemcomitans and P. gingivalis, colonizing the FAI using DNA-probe analysis. Interestingly, the study did not detect the colonization of the screw threads of the abutments. This is in contrast to what was found in the present study, where the threads of the abutments of groups 2 and 3 were colonized with bacteria. This difference may lie in the sample-collection technique. Callan et al. (2005) used paper points for sample collection, whereas in the present study, sterile calcium alginate swabs were used for the microbial sampling. The calcium alginate swabs have a more brush-like appearance compared to the paper points, which may allow a more intimate contact with the threads of the abutment compared to the paper points. In addition, our group used CFUs, whereas Callan et al. (2005) used DNA-probe analysis. This method of direct plating, being less technique sensitive than the DNA-probe analysis, might help explain the differences in regards to the abutment thread sampling.

A recent study showed increases in probing pocket depth, clinical inflammation and numbers of periopathogens seem to indicate that a local bacterial-driven inflammatory reaction may be responsible for the tissue destruction seen at failing implants. In the present study, we tested for microbial colonization of the FAI microgap by A. actinomycetemcomitans and P. gingivalis because both microorganisms have an established role as putative periodontal pathogens. In this context, the bacterial flora associated with peri-implantitis resembles that of chronic periodontitis with significant levels of bacteria such as Fusobacterium spp., Treponema spp., Tannerella forsythia (previously T. forsythensis), Prevotella intermedia, A. actinomycetemcomitans,
and *P. gingivalis*. An FAI that is colonized early by putative periodontal pathogens such as *A. actinomycetemcomitans* and *P. gingivalis* may act as a reservoir of bacteria. This contributes to the establishment and maintenance of microflora that resembles that of chronic periodontitis. In fact Quirynen et al. (2006), using a checkerboard DNA–DNA hybridization and real-time polymerase chain reaction, revealed that a complex microbiota with several pathogenic species was established in peri-implant pockets within 2 weeks after abutment connection. However, the mere presence of putative periodontal pathogens does not indicate a direct etiologic relationship that may lead to a destructive process but may simply indicate a potential pathogenic environment.

It is generally accepted that higher levels of bacteria must be present for extended periods of time to cause tissue damage. However, colonization of periodontal pathogens above threshold levels significantly increased the probability for subjects to have deep pockets or progressive disease. In patients with peri-implantitis, bacterial cell samples were found at stable and diseased implant sites, indicating that a total increase in the bacterial burden was present in patients with peri-implantitis.

A specific microbiological profile has been found comparing healthy and diseased implant sulci. Failing implant sites have demonstrated an infection characterized by microbial species similar to those in periodontitis. In these patients, no distinct differences were seen between healthy and diseased sites. Periodontitis associated microflora was found at stable and failing implant sites in a study by Leonhardt et al. (1999); *Staphylococcus* spp., enterics and *Candida* spp., were found in 55% of the 37 patients with peri-implantitis lesions. This study also showed a significantly higher number of samples positive for *A. actinomycetemcomitans* in
patients with peri-implantitis than in healthy controls. The patients with peri-implantitis were found to have a microbiological profile of adult periodontitis at both healthy and diseased sites. The total bacterial burdens together with other factors such as loading, anatomical and local host-response may contribute to the destructive process in peri-implantitis.

Few studies focused on the decontamination of the inner-implant cavity of two-stage implants. In a recent study, Paolantonio et al. (2008) reported that the application of a 1% chlorhexidine gel in the internal part of the fixture before abutment placement and screw tightening could be an effective method to reduce bacterial colonization over a 6-month period. The authors reported their findings for dental implants with an external hex design that was previously shown to exhibit microbial leakage at the FAI microgap. In addition, Groenendijk et al. (2004) reported that, the internal implant decontamination with 0.2% chlorhexidine solution led to a reduced gingival index and crevicular fluid flow compared to saline treated controls. Although, the clinical impact of bacterial leakage on the implant survival rate seems to be very limited, as shown by longitudinal and cross-sectional studies, the exclusion of bacteria from peri-implant regenerative procedures is considered of paramount importance to obtain clinical success and avoidance of peri-implant disease. Peri-implantitis may have a multifactorial background, however, the increase and maintenance of bacteria in the FAI may prove to show a hyper-inflammatory trait in patients setting off the initiation of tissue destruction around implants in a few patients.

Loading forces on implants may also contribute to the bacterial colonization of the FAI microgap. One disadvantage of the present in vitro study is that loading
conditions were not applied. For instance, in an in vitro experiment using loading forces, Steinebrunner et al. (2005) evaluated bacterial leakage along the FAI microgap and discovered statistically significant differences between five implant systems with respect to the number of chewing cycles and bacterial colonization.\textsuperscript{16} Thus, it is important to confirm or contrast the results of the present study using loading conditions.

The importance of the position, size, and geometry of the implant on marginal bone levels was a subject of various studies demonstrating that several factors are important regarding peri-implant marginal bone loss.\textsuperscript{13, 14, 130, 131} The bacterial colonization of the FAI microgap was reported to be one of these factors. The potential colonization of oral microorganisms of the FAI microgap is presumably impacted by multifactor conditions like the precision fit between the implant components, torque forces when the components are connected, and loading forces when the implants are in function. Indeed, Zipprich et al. evaluated the dynamic behavior of dental implants with different designs of the fixture–abutment connection with respect to microbial colonization. The authors reported the micromovement of the fixture–abutment complex of implants loaded at an angle of 30° when a force of up to 200 N was applied. Interestingly, the same implant system used in our experiment was one of four systems reported to exhibit no micromovement when loaded at 100 N and one of two systems showing no measurable microgap when loaded at 200 N.\textsuperscript{138} The authors speculated that certain implant designs would minimize the pumping effect between the fixture and the abutment, thus preventing bacterial colonization of the FAI interface.

The present study indicated that differences in implant design may affect the potential risk for colonization of oral microorganisms into the FAI microgap. Also, this
study indicated a negligible bacterial penetration down to the threaded part of the FAI of dental implants with a Morse-taper connection.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Dr. Michael Tesmer grew up in Florida, moving from Tampa to Port Richey at the start of high school. After which, he attended Florida Gulf Coast University where he graduated in the spring of 2004. He received his Doctor of Dental Medicine degree in the summer of 2008 from the University of Florida. Michael completed his post-doctoral residency in periodontology at the University of Florida in spring 2011. After graduation, Dr. Tesmer continues to contribute to the field of periodontics through clinical practice, investigational research, academic instruction, and lecturing.