COMPARISON OF TREATMENT PROTOCOLS TO REDUCE THE TOTAL BACTERIAL LOAD IN THE IMPLANT SCREW HOLE

by

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ABSTRACT

Objective: A prospective multi-centre clinical trial was conducted to assess the biofilm inhibitory potential of 1% chlorhexidine (CHX) gel in the internal cavity of implant screw holes, when utilized at the time of surgical implant placement.

Methods: The study included a total of 40 Straumann (S) and Nobel Biocare (N) implants, divided into test (ST or NT) and control (SC or NC) based on the implant system. The implants were placed by two periodontists (private practice) as well as by senior Graduate Periodontics residents at the University of British Columbia. Total colony forming units (CFUs/ml) were assessed 3 months post surgery by means of aerobic and anaerobic culturing and Gram staining was conducted on 15 of the samples. Univariate Mann-Whitney U tests were used to analyze differences between groups (p < 0.001). Gender distribution and sample collection times were compared by means of Fisher’s exact test and Student t-test respectively.

Results: The mean sample collection time was 110 days for the test population and 98 days for the controls (p > 0.05). No statistical differences in term of CFUs/ml were evident when comparing aerobic and anaerobic culturing methods (p > 0.05). The use of 1% CHX gel significantly reduced biofilm formation in both the ST and NT samples when compared with controls (SC and NC) (p < 0.001). Differences between ST and NT did not reach statistical significance (p > 0.05). Overall, 13 implants from the test population demonstrated CFUs/ml below a threshold of 1000 compared to only 1 implant from the control population. Microscopic analysis revealed the presence of mainly Gram-positive coccoid species in 14/15 samples; one sample consisted mainly of rod shaped bacteria.

Conclusion: The application of 1% CHX gel in the internal implant cavity at the time of initial implant surgery greatly reduces the biofilm formation over a 3-month period.
PREFACE

This dissertation is an original intellectual product of the author, Farzan Ghannad. All clinical aspects and associated experimental methods were approved by the University of British Columbia’s Research Ethics Board [certificate number H11-02529].

All Sheep’s blood agar plates were prepared by R. Rolando, Department of Microbiology at the University of British Columbia (UBC). Sample collection tubes were prepared by Dr. Y. Shen, T. Du and Z. Wang in Dr. M. Haapasalo’s microbiology laboratory, in the Department of Oral Biological & Medical Sciences. Farzan Ghannad performed all bacterial culturing, Gram staining and microscopic analysis in the same laboratory. Dr. G. Owen carried out SEM analysis of healing abutment images presented in the “Discussion” section. Sample collection was performed by two UBC affiliated periodontists, Dr. Colin Wiebe and Dr. Farzin Ghannad, as well as senior Graduate Periodontics residents at UBC. Mr. B. Fathinejad prepared all statistical analysis as well as all boxplot figures.
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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>CHX</td>
<td>Chlorhexidine</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CT</td>
<td>Connective tissue</td>
</tr>
<tr>
<td>EBL</td>
<td>External basal lamina</td>
</tr>
<tr>
<td>GBR</td>
<td>Guided bone regeneration</td>
</tr>
<tr>
<td>GI</td>
<td>Gingival index</td>
</tr>
<tr>
<td>HD</td>
<td>Hemidesmosomes</td>
</tr>
<tr>
<td>IAI</td>
<td>Implant-Abutment-Interphase</td>
</tr>
<tr>
<td>IBL</td>
<td>Internal basal lamina</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1β</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>NC</td>
<td>Nobel control</td>
</tr>
<tr>
<td>NT</td>
<td>Nobel test</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PCF</td>
<td>Peri-implant crevicular fluid</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PDL</td>
<td>Periodontal ligament</td>
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<tr>
<td>PI</td>
<td>Plaque index</td>
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<tr>
<td>qPCR</td>
<td>Quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>SC</td>
<td>Straumann control</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SLA</td>
<td>Sandblasted, large-grit, acid-etched</td>
</tr>
<tr>
<td>ST</td>
<td>Straumann test</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor – α</td>
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<td>TRL-9</td>
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1. INTRODUCTION

Rehabilitation of partially or fully edentulous patients with implants is integral to modern dentistry. Studies of dental implants over the years have demonstrated a high predictability for the success rates of implant treatment (Jung et al., 2012). Long-term implant success is greatly dependent on the implant's integration with oral hard and soft tissues. Generally, the initial point of tissue breakdown and surgical trauma is the crestal bone at the implant-tissue interface. Some of the etiological factors suggested for the poor integration of implants are occlusal overload, peri-implantitis, an implant-abutment interphase microgap, impingement of biological width, and the presence of an implant crest module, the transition zone between the implant body and its transosteal region (Oh et al., 2002). While significant attention has been paid to most of the above-mentioned factors, with innovations in implant design and changes to surgical protocols, little has taken place to address the presence of bacteria in the implant microgap or its internal cavity (Figure 25).

The presence of microbiota and their endotoxins in these microgaps, which typically measure no less than 1 µm, have been reported by numerous studies (Harder, Podschan, et al., 2012). Implant manufacturers have attempted to address the microbial presence by reducing the microgap size and/or by shifting the gap away from the bone-implant interphase, the so-called platform-switching concept. The lack of clinical guidelines or surgical protocols recommending the use of antimicrobial agents by the implant manufactures, has forced clinicians to implement empirically derived methods of limiting bacteria in the implant screw hole. Reports of products
being used range from saline or chlorhexidine rinses, polysporin ointments (Johnson & Johnson), or nothing at all in some instances.

This prospective randomized multi-centre clinical trial aims to evaluate the biofilm reduction potential of 1% chlorhexidine gel in the implant screw hole of two bone level implant systems over a period of 90 days, with the objective of recommending a unified antimicrobial protocol to be incorporated for all edentulous rehabilitations with dental implants. Biofilm formation potential of the Straumann (Straumann; Basel, Switzerland) and NobelActive or NobelReplace Conical Connection (Nobel Biocare; Kloten, Switzerland) implants were evaluated. The success of 1% CHX gel was assessed in its ability to reduce CFUs/ml.
2. REVIEW OF THE LITERATURE

2.1 Periodontal Disease

Considered an infectious disease associated with indigenous microbiota, periodontal disease is highly prevalent worldwide (Braga et al., 2010). A recently published study placed the prevalence of periodontitis in the adult US population at 47%, with 30% and 8.5% of this population suffering from moderate or severe periodontitis, respectively (Eke et al., 2012). Periodontitis is a multifactorial disease in which bacterial composition, host immune response as well as genetic components are believed to play a synergistic role. Not only does this disease cause hard and soft tissue degeneration in the oral cavity, but its role in various systemic conditions such as diabetes, cardiovascular disease or even adverse pregnancy outcomes have been substantiated in various studies (Scannapieco et al., 2010). The disease progresses with periods of both exacerbation and remission. While certain types of periodontitis arrest spontaneously and have relatively short lifespans, other types of the disease undergo prolonged periods of tissue destruction, which in severe forms may ultimately lead to edentulism (Armitage et al., 2010). Around natural teeth, the existence of deep periodontal pockets which harbor pathogens have also been shown in a number of studies to act as a reservoir for the colonization of tissues around implants (Leonhardt et al., 1993; Sbordone et al., 1999).

2.2 Dental Implants

2.2.1 Soft-Tissue Attachment

Overall, the attachment of soft tissues to both natural teeth and to implants can be considered to be similar, however differences do exist between the two
attachments, which can dictate the sensitivity or ability of each to resist functional loading, bacterial penetration and the host response. While an implant’s attachment to hard tissue (bone) behaves similarly to an ankylosed tooth's attachment to hard tissue due to the lack of PDL fibers, at the soft tissue level, the implant possesses a junctional epithelium (JE) attachment as well as a connective tissue (CT) attachment.

The JE attachment to the enamel facing the keratinocytes is achieved by the hemidesmosomes (HD) present in the internal basal lamina (IBL), and their fixation on the opposite surface to the CT layer via the external basal lamina (EBL) (Atsuta et al., 2012), creating an Enamel-HD-IBL-EBL-CT connection. This HD attachment is believed to be the main resisting force preventing the apical migration of bacteria around teeth (Borradori and Sonnenberg, 1996).

While past studies reported that implant attachment is similar to the mechanism of tooth attachment, more and more dissimilarities are being reported (Gould et al., 1981; Gould et al., 1984; Klinge, 1991; Fujiseki et al., 2003; Larjava et al., 2011). Current understanding of the peri-implant epithelium suggests that it is a weaker connection in comparison to teeth, and that attachment only truly occurs in the bottom third of the peri-implant epithelium (Ikeda et al., 2000; Atsuta et al., 2012). The biological width around natural teeth is reported to be 2 mm, consisting of 1 mm epithelial and 1 mm connective tissue attachment (Figure 1) (Gargiulo and Wentz, 1961). Around dental implants, the biological width has been reported to be larger, with approximately a 2 mm peri-implant epithelial attachment and 1 mm connective tissue attachment as shown in Figure 2 (Berglundh et al., 1991; Myshin and Wiens, 2005; Larjava et al., 2011).
While on teeth the JE ends at the cement-enamel junction, around implants it always stops at a distance from the gingival margin allowing the formation of a CT attachment to the titanium implant’s surface coronal to the alveolar crest (Berglundh et al., 1991). Collagen fibers present in the connective tissue around implants always extend from the alveolar crest towards the gingival margin in an orientation parallel to the implant itself (Figure 2). The fiber bundles seen around a natural tooth originate from the root cementum and extend in various directions, some attaching to the alveolus, and some to the gingival connective tissue (Figure 1) (Berglundh et al., 1991; Abrahamsson et al., 2002). The morphometric analysis of the CT attachment has also revealed differences between natural teeth and implants. While the CT attachment...
attachment around natural teeth is composed of 63-76% collagen, 5-16% fibroblasts and 3-7% vasculature, the collagen composition is more dense around implants and significantly less vascularized, consisting of 63-86% collagen, 10-33% fibroblasts and only 0.2-2% vasculature (Berglundh et al., 1991; Abrahamsson et al., 2002).

Figure 2. Epithelial (E) and connective tissue (CT) attachment to an implant. Epithelial attachment is longer when compared to natural tooth (Figure 1). Supracrestal fiber (SF) bundles near the CT and implant connection run parallel to the implant surface.

2.2.2 Implant Treatment Success

Implants are widely accepted today as a reliable and successful treatment alternative to a fixed or removable prosthesis for the rehabilitation of partially or fully edentulous patients (Berglundh et al., 2002; Jung et al., 2008; Pjetursson et al., 2012; Romeo and Storelli, 2012). When analyzing the survival of implant treatment, many factors play a significant role such as the timing of implant placement, the quality of
the bone, the size of the implant including both its length as well as its width, the
timing of the implant loading, the patient’s history of periodontal disease or other
modifying factors, and the type of prosthetic restoration. Numerous studies as well as
review papers have looked at these factors individually or in combination. The
survival rate has been found to range between 95.8-100% over a period of 6-43
months for immediately loaded implants with higher survival rates associated with
placements in the mandible (Strub et al., 2012). The 5 and 10 year survival rate for
implants supporting single crowns has been reported to be 97.2% and 95.2%,
respectively, in a recently published meta-analysis (Jung et al., 2012). Even when
comparing the success of implants placed in sites treated with guided bone
regeneration (GBR), the results were slightly less favorable for the grafted sites,
however the results were not statistically significant (Bazrafshan and Darby, 2013).
The timing of complications as well as loss of certain implants varies depending on
the factors mentioned above. A systematic review by Berglundh et al. (2002) also
looked at the timing of complications and concluded that implant loss prior to
functional loading (“early”) can be expected to occur in 2.5% of all implants placed
(Berglundh et al., 2002). Fixed prosthetic constructions lead to the loss (“late”) of 2-
3% of the implants, while >5% of the implants in an overdenture therapy failed within
a period of 5 years (Berglundh et al., 2002). A second study with longer follow up
times of 9-14 years and a population size of 218 individuals with 1057 implants
yielded similar results (Roos-Jansåker et al., 2006a). With a total loss of 46 implants
(4.4%), 2.7% occurred prior to placement of the suprastructure (“early”) while 0.7%
were lost within the first year of loading, and thereafter an additional 1% were lost (Roos-Jansåker et al., 2006a).

2.2.3 Peri-Implant Disease

Regardless of the high success rate of implants, they are not immune from complications. These complications can be caused by poor treatment planning, surgical or restorative inexperience of clinicians, as well as material and bacterial influences.

Gingivitis and periodontitis are two terms used to describe patients’ immune responses and subsequent tissue inflammation and destruction affecting teeth and their surroundings. While the term “gingivitis” refers to inflammation of the soft tissue without any loss of supporting tissue, "periodontitis" is the combination of soft tissue inflammation in conjunction with the loss of attachment and supporting bone. In 1994, two additional terms were introduced at the first European Workshop on Periodontology. "Peri-implant mucositis" was defined as the reversible soft tissue inflammation surrounding a functioning or osseointegrated implant; “peri-implantitis” is descriptive of inflammation that has lead to the loss of supporting bone around a functioning implant beyond the scope of remodeling associated with healing (Albrektsson and Isidor, 1994).

Löe et al. (1965) provided the evidence to support the cause-and-effect relationship between plaque accumulation and gingivitis in his “Experimental Gingivitis” study (Löe et al., 1965). In a clinical study in 1994, Pontoriero et al. provided the evidence linking plaque as a causative factor in peri-implant mucositis with the same methodology as Löe et al. (1965). After 20 partially edentulous patients
were given full periodontal treatment and oral implant treatment in the posterior areas, the patients were asked to refrain from oral hygiene practices at the implant sites as well as at the adjacent teeth, for a period of three weeks (Pontoriero et al., 1994). All parameters such as gingival index (GI), plaque index (PI) and probing depth (PD) showed a statistically significant increase when compared to the baseline results in both the peri-implant as well as the tooth sites (Pontoriero et al., 1994). The mean GI values of 0.2 and 0.5 at baseline for the test and control sites increased to 1.6 and 1.9, respectively, after three weeks. Mean PI values increased from 0.5 and 0.8 at the implant sites and from 0.3 and 0.4 at the tooth sites to a mean PI value of 2.4 and 2.0, respectively, in the same time period (Pontoriero et al., 1994). Periodontal probing depths also increased at these sites from 2.4 and 2.8 mm at baseline to 3.7 and 3.1 mm, respectively, as a result of soft tissue inflammation (Pontoriero et al., 1994).

The prevalence of peri-implant mucositis has been reported at 48-50%; however due to its reversible nature, it is possible that it is under-diagnosed (Roos-Jansåker et al., 2006b; Zitzmann and Berglundh, 2008).

The correct diagnosis of peri-implantitis is even more complex than that of peri-implant mucositis, with different criteria and thresholds being used by different authors and the reported incidence and prevalence varying significantly from publication to publication. The prevalence of peri-implantitis has been reported to be 6.61% (Roos-Jansåker et al., 2006b), or 23% (Marrone et al., 2012) or 36.6% (Koldsland et al., 2010) just to name few.
2.2.4 Peri-Implantitis Differences from Periodontitis

Although peri-implantitis and periodontitis appear to share common etiological factors such as common bacterial species (Heitz-Mayfield and Lang, 2010) and similar inflammatory responses (Javed et al., 2011), the rate of destruction appears to be much greater in the peri-implant sites (Lindhe et al., 1992; Schou et al., 1993). This increased rate of progression could be due to the lack of inserting collagen fibers into the implants (Schou et al., 1993; Abrahamsson et al., 2002). It was also noted that in ligature-induced peri-implantitis, ligature removal did not stop the progression, but rather a continuous progression of the disease and its subsequent bone loss was recorded (Albouy et al., 2008; Albouy et al., 2009). This loss was believed to be directly associated with the biofilm formed on the implant surface, which is the primary target in the treatment of peri-implantitis or peri-implant mucositis. However, other evidence suggests a major difference between periodontitis lesions, which are always encapsulated by a connective tissue barrier that prevents the infiltration of the bone marrow by inflammatory cells in contrast to the peri-implantitis lesions (Seymour et al., 1979; Heitz-Mayfield and Lang, 2010). Peri-implantitis lesions can allow the infiltration of such inflammatory cells into the bone marrow unimpeded by the healthy connective tissue; hence the rapid or continuous progression of the disease (Lindhe et al., 1992; Marinello et al., 1995; Heitz-Mayfield and Lang, 2010; Berglundh et al., 2011). Histological studies of these peri-implantitis sites in animals showed increased osteoclastic activity and the presence of Howship’s Lacunae at the bone crest (Marinello et al., 1995).
While examining human soft tissue biopsies from peri-implantitis and periodontitis sites, similarities and dissimilarities were noted on a histopathological level (Berglundh et al., 2011). In general, the inflammatory cell infiltrates would extend apical to the pocket epithelium at peri-implant sites, in contrast to periodontitis (Berglundh et al., 2004). While the proportion of plasma cells and lymphocytes were fairly high yet similar in both conditions, a higher proportion of macrophages and neutrophil granulocytes were present at diseased implant sites. Contradictory results regarding the proportion of B cells and T cells exist, with older studies reporting a higher ratio of B cells to T cells in peri-implantitis lesions (Gualini and Berglundh, 2003) while the opposite is being reported by more recent publications (Bullon et al., 2004; Berglundh and Donati, 2005; Kim et al., 2010).

Levels of osteoclast-regulating cytokines of soft tissue samples obtained from diseased implant sites, from chronic periodontitis sites as well as from controls were compared using immunohistochemical analysis. Higher proportions of IL-1α and IL-6 cytokines were reported for peri-implant lesions while lower proportions of TNF-α in comparison to those found in tissues affected by chronic periodontitis (Konttinen et al., 2006).

In an attempt to measure the enzymatic activity of elastase, as well as to measure lactoferrin and IL-1β concentrations at different sites, Hultin et al. (2002) collected crevicular fluid samples from both diseased implants as well as stable implants and compared them to samples collected from healthy teeth and sites with periodontitis (Hultin et al., 2002). Both lactoferrin and elastase levels were increased at the peri-implantitis sites when compared to the levels at the sites of stable implants.
and at healthy teeth in the same patients. While Hultin et al. (2002) reported that samples from diseased teeth did not differ from healthy teeth or from stable implant sites, the majority of authors have reported an increased elastase activity in the GCF of periodontitis sites when comparing them to healthy teeth sites (Palcanis et al., 1992; Meyle et al., 1992; Chen et al., 2000). IL-1β concentrations were similar in all samples tested and did not show any statistical differences between the groups (Hultin et al., 2002).

2.2.5 Periodontitis Associated Peri-Implantitis

Research has shown that susceptible or periodontally affected individuals who do not receive proper treatment have an increased risk of suffering from loss of attachment around their teeth as well as their implants (Hardt et al., 2002). When implants were examined one year after placement in susceptible individuals, the conditions found around the implants correlated to the overall oral image and periodontal conditions (Brägger et al., 1997).

After 10 years of supportive periodontal therapy at 3-6 month intervals, overall implant survival rates for teeth which had been lost due to chronic periodontitis was 90.5% vs. 96.5% for those lost for other reasons (Karoussis et al., 2003). This increased susceptibility only became evident after a minimum of six years of implant function (Karoussis et al., 2003). Periodontitis patients appear to have excellent short-term results, however drops in success become evident during the 5-10 year period (Schou et al., 2004).

A more recent systematic review and meta-analysis conducted by Safii et al. (2010) looked at the marginal bone loss around implants as well as the risk for
implant failures in patients with a history of periodontal disease. Their analysis favored periodontally healthy individuals for implant survival with an odds ratio (OR) of 3.02 and demonstrated more peri-implant bone loss (standard mean difference = 0.61 mm) in those with a history of periodontitis (Safii et al., 2010). When studying peri-implantitis susceptibility after periodontal therapy and active supportive care, Pjetursson et al. (2012) concluded that residual pockets of ≥5 mm after active treatment represented a significant risk factor for the development of peri-implantitis or even the loss of the implant (Pjetursson et al., 2012). Furthermore, patients who develop re-infection while undergoing supportive periodontal therapy (SPT) had an increased risk when compared to periodontally stable individuals (Pjetursson et al., 2012).

Although lower success rates have been reported for patients with a history of periodontitis when compared to healthy individuals, the success rates still remain within an acceptable range and the differences are usually not considered statistically significant (Schou et al., 2004; Karoussis et al., 2007).

2.3 Bacteria

2.3.1 Periodontal Pathogens

The microorganisms inhabiting the oral cavity have in the past been referred to as oral microflora or microbiota. More recently the term microbiome was introduced by Joshua Lederberg “to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease” (Lederberg and Mccray, 2001). The etiology of periodontitis is considered to be polymicrobial (Griffen et al., 2012)
and the infection with specific microorganisms is essential for disease initiation and progression (Haffajee and Socransky, 1994; Laine et al., 2013). Estimates place the number of bacterial species inhabiting the oral cavity at about 500 to 700 (Socransky and Haffajee, 1994; Wilson et al., 1997; Aas et al., 2005), while only a subset are believed to be opportunistic pathogens (Paster et al., 2001). Most putative periodontal pathogens are Gram-negative anaerobic rods. In addition, herpes viruses have the ability to reduce host defense capabilities while indirectly inducing the overgrowth of pathogenic bacteria (Slots, 2010). In 1998 using whole-genomic hybridization, *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia* were classified as showing a strong association with periodontal disease and each other (Socransky et al., 1998) while the association of *Prevotella intermedia, Peptostreptococcus micros* and *Aggregatibacter actinomycetemcomitans* was confirmed thereafter (Laine et al., 2013).

Using a 16S rRNA-based PCR test to look at the carriage rate of periodontal pathogens in the saliva of 8028 subjects who were already part of a national health survey, a detection rate of 56.9% was found for *T. forsythia*, 38.2% for *T. denticola*, 35.4% for *P. gingivalis*, 31.3% for *C. rectus*, 20.0% for *Aggregatibacter actinomycetemcomitans*, and 13.9% for *P. intermedia* (Könönen et al., 2007). In 88.2% of the study population, at least one of the above mentioned pathogens was detected (Könönen et al., 2007).

Using 16S pyrosequencing, Griffen et al. (2012) compared the bacterial composition of both periodontal health and disease. Their results confirmed earlier findings by Socransky et al. (1998) in regard to the pathogenicity of *P. gingivalis, T.*
denticola and T. forsythia; however, they found that T. forsythia was only the sixteenth most prevalent pathogen in diseased sites while Filifactor alocis was as prevalent as the first two (Griffen et al., 2012). Pyrosequencing also enabled them to examine different bacterial phyla and link those from the family of Spirochaetes, Synergistetes and Bacteroidetes to disease (Griffen et al., 2012). The authors also attempted to compare their sequencing results to those of cultivation; they realized that it is not possible to cultivate over half of the bacteria associated with periodontitis while this is only true for a third of the species associated with periodontal health (Griffen et al., 2012).

Not all species identified in individuals are considered pathogenic; a subset of species have also been identified to be “beneficial”, Veillonella parvula, Actinomyces sp., Streptococcus sanguis, Streptococcus mitis and Streptococcus intermedius are few such organisms. Clusters have been observed at sites with less active disease (Feres et al., 2004), such sites also responded more favorably to treatment.

The summation of the antagonistic effects caused by such beneficial oral microbiota prevents the colonization of the periodontal pocket by opportunistic exogenous and/or endogenous pathogens. Development of periodontal disease or other microbiome-dependent oral pathology is as a result of disruption between the harmonic interaction of the host and its commensal microorganisms (van Essche et al., 2013). Such disruptions and the resulting inflammatory response by the macrophages and monocytes to the lipopolysaccharides of the pathogens initiates the production of pro-inflammatory cytokines which may lead to the breaking down of periodontal or peri-implant tissue (Cosgarea et al., 2012).
2.3.2 Oral Biofilm

Oral biofilms, also known as dental plaque, are defined as “matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces” (Costerton et al., 1994). They form on all non-shedding surfaces in a fluid system (Lang et al., 2000). Their development is influenced by numerous factors such as the availability of oxygen, nutrients, environmental pH and most importantly their micro-organismal co-adhesion (Socransky and Haffajee, 2002; Filoche et al., 2009). In the oral cavity, saliva is the provider of all the necessary proteins and bacteria for the formation of a biofilm, as well as facilitating their transport (Scannapieco, 1994). It has been estimated that 1 ml of saliva can contain up to $10^9$ bacteria (Dawes et al., 2001).

The process is initiated by the formation of pellicle on the tooth surfaces which provides the specific receptors for attachment of the initial colonizers of streptococcal species (Darveau et al., 1997). According to Aas et al. (2005), certain microorganisms have a preference for tooth surfaces such as *R. dentocariosa, Actinomyces spp., S. sanguinis, S. gordonii, and A. defective* (Aas et al., 2005) and are considered to belong to the initial colonizer family. As the biofilm develops and its environment transforms from aerobic to anaerobic, its bacterial composition also shifts from being dominated by Gram-positive coccoid species to a larger proportion of Gram-negative bacteria, motile rods and spirochetes (Feres et al., 2004). This micro-organismal composition shift is representative of a clinical transformation from a healthy periodontium to diseased (Listgarten and Helldén, 1978; Xie et al., 2000).
Colonizing species in a biofilm benefit from a number of advantages associated with such co-adhesion. While being protected from competing microorganisms, host defenses and potentially toxic environmental substances such as antibiotics, which make their elimination extremely difficult, biofilms have the additional benefit of cross-feeding (nutrition provided by one species for another) as well as removal or utilization of harmful metabolic products of one organism by another (Socransky and Haffajee, 2002; Costerton et al., 2005).

2.3.3 Implant Biofilm

Scanning electron microscopy (SEM) of oral implant surfaces appears to show a similar biofilm formation pattern as seen on natural teeth (Lang et al., 2000). Furthermore it has also been established that the microbiota associated with gingival health or gingivitis are similar or closely resemble those associated with healthy peri-implant sulcus or mucositis (Lang et al., 2000; Shahabouee et al., 2012), while the composition of peri-implantitis sites resembles more the pockets of severe chronic periodontitis (Lang et al., 2000); these results were obtained through microbial culturing. More reliable methods of bacterial identification such as PCR testing and better primers have given us a better understanding of population differences when comparing implants to teeth. With the exception of A. actinomyctemcomitans and P. gingivalis which have a similar frequency of occurrence, all other bacterial species as well as the red complex bacteria have a higher frequency of detection in periodontitis than peri-implantitis (Cortelli et al., 2013). There is an overall tendency to a higher frequency of bacteria in periodontal sites, with a population increase from health to gingivitis to periodontitis when compared to peri-implant sites (Cortelli et al., 2013).
While some authors believe the bacterial species of the two hard-tissue diseases to be similar even if with different proportions (Cortelli et al., 2013; Lang et al., 2000), Heuer et al. (2012) found that certain bacterial genera were present at several teeth sites but not at implant sites (Heuer et al., 2012). Using a broad-range PCR technique, the investigators were able to identify 20 different genera at both sites; 19 different sequences were identified at teeth sites exclusively while only six were at implants (Heuer et al., 2012). While the study only looked at one implant system, it does imply that the bacterial composition can be a consequence of the surface differences between teeth and implant sites or of the anatomical variations of mucosal or gingival tissues (Heuer et al., 2012).

In a prospective, cross-sectional study of 50 implants placed in partially edentulous periodontally healthy patients, bacterial samples were collected from the sulci of implants (≤ 4mm) and compared to 34 similar teeth sites (Shahabouee et al., 2012). The results indicated the presence of six groups of anaerobic bacteria at both sites with insignificant variation in the frequency of occurrence favoring teeth sulci. Gram-positive cocci had the highest frequency of detection in both groups while other detected bacteria were *P. gingivalis*, *Prevotella*, Gram-negative cocci, *Fusobacterium*, and *B. fragilis* (Shahabouee et al., 2012). The results found in this study confirm those from the past that healthy sulci in both implant sites as well as teeth resemble one another, however the emphasis being on the depth of the sulci (≤ 4mm) (Shahabouee et al., 2012).
2.3.4 Implant Surface Colonization

Initial studies suggested that two weeks following the placement of a transmucosal implant in edentulous patients with no history of periodontal disease, a predominantly Gram-positive bacterial population resembling those associated with gingival health or gingivitis was detected in the peri-implant sulcus (Mombelli et al., 1988; Lang et al., 2000). Periodontal pathogens, which had colonized the residual periodontal pockets, were also present in the peri-implant sites three months after transmucosal placement (Lang et al., 2000). More recent data suggested that such colonization occurs even faster and the presence of pathogens could be verified within 10-14 days after implant placement (AL De Boever and JA De Boever, 2006; Quirynen et al., 2006). A study conducted by Fürst et al. (2007) looked at the development of sub-gingival microbiota at implant sites and compared this to the development of sub-gingival microbiota near teeth. Colonization of the implant surface as early as 30 minutes after surgical placement was reported and a steady increase in the bacterial load was observed over a 12 week period (Fürst et al., 2007). Although the bacterial load was significantly higher at the teeth sites (Salvi et al., 2008; Fürst et al., 2007), the study demonstrated that a sub-mucosal implant microbiota is already established by the time the surgical procedure is completed (Fürst et al., 2007).

When examining the so called “pristine pockets” at implant sites, namely pockets created after a submerged implant is uncovered and a transmucosal healing abutment is placed, Quirynen et al. (2005) demonstrated that certain pathogens were present more often if they were also be detected in the pockets of adjacent teeth,
further highlighting the significance of the supragingival environment (Quirynen et al., 2005).

The ability of bacteria to adhere to the implant surface is not only affected by the surface roughness, but the implant's surface free energy also facilitates biofilm formation (Teughels et al., 2006; Subramani et al., 2009). Adhesion potential of bacteria used to correlate with surface roughness; however a recent in vitro study comparing the formation of biofilm on different implant surfaces ranging from machined implant surfaces to sandblasted/acid-etched (SLA) surfaces concluded that although the modified SLA surface showed the greatest degree of biofilm formation, unexpectedly the differences to the machined surface implants were not significant (Schmidlin et al., 2013). Certain bacteria appear to have a higher affinity for the titanium surface rather than for the tooth surface, such as S. aureus (Harris and Richards, 2006). The formation of a bacterial biofilm on medical devices is considered an important step prior to infection (Costerton et al., 2005).

In a novel study design, Elter et al. (2008) investigated the effect of different surface treatments on supra- and subgingival biofilm formation through SEM analysis of healing abutments. The authors pointed out that most clinical examinations published to date have only looked at the presence of planktonic bacteria in the peri-implant tissue. The outer surface of 15 Nobel Biocare healing abutments were divided into quadrants and three of the sections were treated either by sandblasting, by acid-etching, or by grinding the surface with a diamond bur in order to obtain different levels of roughness (Elter et al., 2008) while the last quadrant served as the untreated control. The abutments were all cleaned in an ultrasonic bath and inserted on the
implants; in order to have an exact idea where the border lies between supra- and subgingival region, silicone impressions were taken from the abutments. Patients were instructed to continue their regular hygiene habits while not using any antimicrobial rinses for a period of 14 days; then abutments were removed and after rinsing with sterile water, they were air dried (Elter et al., 2008). Surface roughness measurements under the SEM resulted in a mean Ra of 0.9 µm for the sandblasted quadrant followed by 0.4 µm for the ground area and 0.3 µm for the surface treated by acid-etching; the untreated control surface was 0.2 µm (Elter et al., 2008). Biofilm analysis showed that the highest surface area coverage was 47.4% ± 32.4% supragingival and 1.3% ± 1.2% subgingival on the sandblasted surface, followed by the ground surface and finally on the acid-etched surface, 15.6% ±18.7 and 0.6% ± 1.3% were covered, respectively (Elter et al., 2008). The control surface presented the lowest biofilm formation, 5.7% ± 14.4% and 0.6% ± 1.2%. While supragingival results were significantly different, subgingival differences were not (Elter et al., 2008). The authors attributed the low subgingival biofilm formation to the presence of a tight peri-implant seal formed by hemidesmosomal attachment, collagen fibers and other anatomical factors and pointed out that the increase in supragingival plaque accumulation did not contribute to its subgingival formation (Elter et al., 2008).

It has been reported that below a certain surface roughness threshold (Ra 0.2 µm), the microbial attachment potential does not decrease any further; therefore a Ra of 0.2 µm serves as the ideal surface texture for both obtaining soft tissue attachment and reducing microbial adhesion (Bollen et al., 1996; Subramani et al., 2009). As
reported in the Elter et al. (2008) study, the surface roughness of the control was measured as exactly this number.

2.4 Internal Colonization

The colonization of the peri-implant environment can occur within minutes of implant placement as previously mentioned (Fürst et al., 2007). In addition to the peri-implant tissue, microorganisms may leak into the implant-abutment interface (IAI) (Quirynen and van Steenberghe, 1993; Quirynen et al., 1994) or remain trapped inside the implant screw hole as a result of their exposure to the oral cavity at the time of placement (Aloise et al., 2010; Faria et al., 2011; Tripodi et al., 2012), causing an area of inflammation at the implant-abutment-bone junction as a result of the bacterial reservoir created at the site (Ericsson et al., 1995; Broggini et al., 2003; Broggini et al., 2006). The microbial leakage into the IAI increases further as result of functional loading (Steinebrunner et al., 2005; Koutouzis et al., 2011).

When trying to identify bacteria present in the internal cavity of the implant screw hole, a distinction between the bacteria in a biofilm and the non-adherent microbiota needs to be made. Recently, Harder et al. (2012) conducted a study with a major difference to all other previously reported publications. They incorporated two different methods of sampling in order to collect both the non-adherent microbial and biofilm samples (Harder, Podschun, et al., 2012). Overall, 35 two-piece implants were analyzed to accurately detect the intra-implant microflora. For the non-adherent microbial collection, a syringe filled with 100 µm sterile NaCl solution was used to first fill the implant screw hole after the abutment removal followed by collection of the NaCl back into the syringe; biofilm samples were collected using sterile
microbrushes (Harder, Podschun, et al., 2012). The significance of their findings was not that 103 different species were detected, but rather that 27 and 33 of the species were exclusive to samples collected from the biofilm and non-adherent microbial samples, respectively (Harder, Podschun, et al., 2012). The remaining forty-three species were present in both samples (Harder, Podschun, et al., 2012).

2.4.1 Outward Bacterial Leakage

The origin of the microorganisms contaminating the internal components of implants has been debated for years. Studies conducting a variety of testing have been able to show that the microbial leakage can travel in both directions, towards the internal cavity of the implant from the peri-implant tissue or as a result of being trapped on the inside where they proliferate and leak towards the peri-implant tissue.

In order to study the possibility of microbial “outward” leakage from inside the implant to its outer surface and surrounding, a total of twenty implants from two different manufacturers were used, 10 in each group (Aloise et al., 2010). Due to its small size of 0.5 to 1.0 µm, 0.1 µl of S. sanguinis biotype II was selected to inoculate the internal component of the implants in both groups followed by the placement of the abutments and torqueing according to the manufacturer's instructions (Aloise et al., 2010). Following surface testing of the implants to exclude any contamination, all implants were submerged in a nutrient rich solution (BHI) for a period of 14 days and incubated at 37°C under anaerobic conditions (Aloise et al., 2010). The clarity of the solution was examined on a daily basis and if the BHI appeared cloudy, 1 µl of the solution was plated on a BHI-Agar base and cultured for S. sanguinis. Two cases of bacterial leakage were detected in each group (20%); all cases occurred on the second
day of incubation while no additional leakage was noted for the remaining duration of the study (Aloise et al., 2010). Earlier studies using the same tapered connection found the same phenomenon to be true, however at a slightly higher rate (50%), which according to the authors could be due to lack of appropriate torquing of the abutments (Jansen et al., 1997).

A more recent study by Tripodi et al. (2012) compared the outward leakage of internal hexagon versus morse-taper implant-abutment connections using 0.1 µl of *A. actinomycetemcomitans* which has an average size of 0.4 × 1.0 µm, in half of the samples, and the other half of the same group was inoculated with 0.1 µl of *Pseudomonas aeruginosa*, an aerobic/facultative anaerobe rod-shaped motile bacterium with a size ranging from 0.5 to 1.0 µm wide and 1.5 to 5.0 µm long (Tripodi et al., 2012). Following the same protocol used by Aloise et al. (2010), a total of two implants from the morse-taper group and five from the hexagonal group demonstrated bacterial leakage after a total of 28 days of monitoring (Tripodi et al., 2012). Authors pointed out the 22 days delay before leakage was recorded in the tapered group, which has been a weakness in previous studies with significantly shorter study periods (Tripodi et al., 2012).

Regardless of their origin, one fact remains; the biofilm formed as a result of the microbial leakage proliferates in the implant-abutment microgap and causes peri-implant inflammation (Broggini et al., 2006).

### 2.4.2 Influence of Repeated Abutment Dis-/Reconnection

Studies on the protective properties of soft tissue attachment have attempted to prove its significance to implant success. The effects of repeated abutment removal
and its subsequent reattachment on the peri-implant tissue and bacterial migration were examined by Abrahamsson et al. (1997) in an experimental dog study. One implant was placed in each quadrant of the mandibles of five beagle dogs. After a three-month healing period, a six-month period of experimentation started with stringent plaque control. The healing abutments of the right quadrant were removed on a monthly basis and cleaned and subsequently they were reattached; this process was repeated a total of five times and at the end of the sixth month period, all dogs were sacrificed and block resection was carried out (Abrahamsson et al., 1997). Histometric analysis revealed statistical differences between all measurements. At the control sites, the junctional epithelium was about 2.04 mm long and the CT was 1.28 mm; the crestal bone position was about 0.78 mm apical to the implant-abutment junction (Abrahamsson et al., 1997). In comparison, the test implants presented with shorter epithelial and CT attachment, 1.65 mm and 0.85 mm respectively, while at the same time, the crestal bone was positioned even further apically at a level 1.5 mm from the implant abutment junction (Abrahamsson et al., 1997). Overall the test sites suffered greater soft tissue recession when compared to control abutments (Abrahamsson et al., 1997).

Numerous other studies have also looked at the effect of reduced abutment removal on the healing of soft and hard tissues around implants. In a clinical study on immediately placed implants, ten received provisional abutments (PA) and fifteen received definitive abutments (DA); after final restoration, radiographs were taken from all the implants using a standardized method at baseline ($T_0 = $ implant insertion), restoration ($T_1 = 3$ months), 18 months ($T_2$) and 3 years ($T_3$) (Canullo et al., 2010).
The results of subtraction radiography demonstrated significant differences at time \( T_2 \) (0.1 mm) and \( T_3 \) (0.2 mm) favoring the DA group in regards to decreased marginal bone resorption (Canullo et al., 2010). Overall, the control group lost about 0.55 mm after three years while the test group only lost 0.34 mm over the same period (Canullo et al., 2010). Similar conclusions in regard to the effect of abutment nonremoval at the time of implant placement were reported by Degidi et al. (2013) using a cone beam computer tomography as the method of analysis over a 24 month period (Degidi et al., 2013).

In a split-mouth animal study, histometric analysis further confirmed the previous results. Twelve implants in three dogs with two different types of abutment materials (titanium and zirconium oxide) served both as tests and controls. The test abutments were dis-/reconnected at four and six weeks while the controls were left undisturbed for the entire eight weeks, followed by block dissections (Becker et al., 2012). The alveolar bone crest was significantly lower to the most apical portion of the JE as well as the implant shoulder in all test samples and no significant difference was reported in regard to different abutment material (Becker et al., 2012).

2.4.3 Influence of Load

In an \textit{in vitro} dynamic loading model study of two different implant-abutment connections by Koutouzis et al. (2011), a total of twenty-eight implant-abutment microgaps were assessed on their ability to prevent microbial leakage. Group 1 comprised fourteen \((n = 14)\) internal morse-taper connections while group 2 included equal numbers of implants with a four-groove conical internal connection (Koutouzis et al., 2011). All implants were submerged in an \textit{Escherichia coli} bacterial solution
and loaded with 15 N over 500,000 cycles. Culturing of the microbial samples collected from the abutment threads revealed penetration of *E. coli* in only one of the implants in group 1, however 12 out of the 14 abutments in group 2 were contaminated (Koutouzis et al., 2011), a strong indicator of the influence loading as well as implant-abutment design can have on such leakage (Koutouzis et al., 2011).

To further substantiate their findings, in a more recent study with a larger sample size and increased loading force of 50 N, Koutouzis et al (2012) compared forty morse-taper implants in two groups of 20 under non-loading (group 1) and loading (group 2) conditions. While only one implant in the non-loading group was penetrated by *E. coli*, four of the loading group implants tested positive for the bacteria (Koutouzis et al., 2012). Furthermore, while the maximum number of total CFU’s in the non-loading group only reached 60, the maximum value in the loading group was 200, a statistically significant difference (Koutouzis et al., 2012). Similar results were found in the past under different experimental loading conditions (Steinebrunner et al., 2005). Increased loading generates micro-movement and in turn increases the implant-abutment gap causing fluid as well as microorganisms to move to the inner parts of implants (Khraisat et al., 2006).

Slight increases are all that is necessary to further facilitate bacterial movement from the internal components to the outside and vice-versa, since the average size of oral bacterial species ranges between 1.1-1.5 µm in length and 2 - 6 µm in diameter with even smaller species such as spirochetes having a diameter of 0.1 - 0.5 µm (Jansen et al., 1997). The trapped bacteria are believed to develop into biofilms with varying composition that impact the peri-implant health (Quirynen et al., 2002).
Implant-abutment design changes by the manufacturers have attempted to achieve two main goals: reduce the mechanical stress caused by micro-movement at the implant-abutment-bone interface or at the very least shift this stress away from the site, and limit the gap dimensions at the IAI and thereby reduce bacterial leakage potential. Both of these have been addressed with the “platform-switching” implant designs.

2.4.4 Influence of Implant Design

Different implant designs have been shown to have differing abilities to block bacterial leakage, although no differences were recorded between an internal hexagonal system versus an external design (Jansen et al., 1997; Steinebrunner et al., 2005). The morse-taper design is not only more efficient in reducing bacterial passage, but it also has a superior mechanical force distribution from the prosthesis to the implant and so can maintain a stable torque and abutment, and subsequently keep the supporting tissue stable (Merz et al., 2000; Aloise et al., 2010; Koutouzis et al., 2011; Schmitt et al., 2013).

While testing six different abutment-implant connection on their ability to block the penetration of *S. sanguinis*, the authors confirmed previous findings that no implant-abutment connection can completely block bacterial leakage (Ricomini Filho et al., 2010). The only implant abutment construction shown in a study by Assenza et al. (2012) to prevent bacterial leakage when compared to both a trilobed and internal conical connection was a cement connected implant abutment (Assenza et al., 2012); however abutments of this type are difficult if not impossible to remove. Furthermore, excess cement has been known to cause far more severe inflammatory reactions at
peri-implant tissue sites (Korsch et al., 2013) than the endotoxins from trapped bacteria.

2.4.5 Implant-Abutment Microgap & Leakage

Misfits of laboratory-made custom abutments and implants by different manufacturers are believed to significantly increase the vertical and horizontal microgaps while resulting in undesirable micro-movement of the implant-abutment-interphase leading to an increase of microbial leakage and stress on the crestal bone.

Analyses of the microgap and implant-abutment misfits were carried out by Meleo et al. (2012) using micro-tomography (micro-CT). They looked at three different implant-abutment connections, the Ankylos, the Straumann bone level, and the Bicon systems, with each sample being imaged five consecutive times at predefined magnification and cross-section sizes. However, due to their limited resolution acquisition of 10 µm, a major limitation of their study, no microgap was detected in any of these non-loading systems; therefore the investigators concluded that if microgaps were present, they were smaller than the 10 µm threshold (Meleo et al., 2012).

While the previous authors failed to demonstrate the presence of a microgap, Rack et al. (2010) using synchrotron-based micro-imaging attempted to demonstrate the presence of such gap under non-load bearing as well as load bearing conditions on a Straumann bone level implant. After torqueing of the healing abutment with a force of 25 Ncm, the construction was imaged under force application (0, 30, 60, 100 N) at a 90° angle. A microgap was detectable under all force variations, with the smallest parallel walled gap being recorded without any mechanical load (0 N), >1 µm and less
than 4 µm (Rack et al., 2010). At 30 N, the gap was about 4 µm, 11 µm at a force of 60 N and finally at the 100 N force, the gap was no longer parallel walled and measured 22 µm at its entrance and 15 µm at its base (Rack et al., 2010).

Da Cunha et al. (2012) using SEM imaging examined the effects of Procera abutments on vertical implant-abutment gap changes in the Nobel Biocare system, the implant the Procera was originally designed to be used for, as well as two other widely used implant systems. The three groups each consisting of eight identical Procera abutments were tightened with a torque of 35 Ncm. The Nobel Biocare group had the lowest mean misfit of 5.7 ± 0.39 µm and was statistically smaller than the other two tested systems, one measuring 9.53 ± 0.53 µm and the second one measuring 10.62 ± 2.16 µm (de Morais Alves da Cunha et al., 2012). Knowing the significantly smaller dimension of pathogens and their respective endotoxins, variations as a result of such misfits can further increase bacterial penetration into the implant-abutment microgap. Misfit values have been reported to range between 2 to 7 µm in the same implant system (Piattelli et al., 2003), demonstrating a high variability even within its intended use.

One study looked at the inclusion of a polymeric washer placed between the parts of the implant system. Such methods have been able to reduce the internal contamination of implant systems; however, in this study complete elimination was not achieved (Rimondini et al., 2001).

2.5 Inflammation at Implant-Abutment-Bone

Increased numbers of inflammatory cells have been reported at the IAI or slightly coronal to it on the soft tissue around this junction (Broggini et al., 2003) and
the resulting failure of peri-implantitis treatment has been blamed on the presence of bacteria and their proliferation in this gap (Broggini et al., 2003; Broggini et al., 2006). Furthermore, the presence of such microbial leakage could result in marginal bone loss caused by the inflammatory infiltrate at these sites (Hermann et al., 2001).

Molecular leakage from conical implant-abutment connections was tested by Harder et al. (2009) in a study using AstraTech (n = 8) and Ankylos (n = 8) implants, *S. enterica* lipopolysaccharides served as the test endotoxin. After inoculation of the deepest part of each implant system with 0.5 µl of the endotoxin solution, abutments were tightened according to the manufacturer's recommendation (Harder et al., 2009). Samples collected at baseline, 5 min, 24, 72 and 168 hours were compared to control samples. While all samples in the Ankylos group showed signs of contamination just after 5 min of inoculation with the supernatant solution, only five of the AstraTech group contaminated the solution within the same time period; however after 168 h, only one AstraTech implant had not contaminated the solution (Harder et al., 2009). Difference between the two systems was significant in both the extent of contamination as well as the number of implants; however a constant increase over time in all samples was true for both groups (Harder et al., 2009).

To further investigate the negative influence of bacterial reservoir at the IAI, Herder et al. (2012) assessed the lipopolysaccharide (LPS) microleakage at this interphase and the induced pro-inflammatory cytokine production (Harder, Quabius, et al., 2012). LPS, endotoxins that reside on the outer membrane of Gram-negative bacterial are considerably smaller than bacteria themselves (Caroff et al., 2002), the microgap not only needs to be small enough to prevent bacterial microleakage, but
also prevent the leakage of such endotoxins (Harder, Quabius, et al., 2012). After inoculation of fourteen implants from two different manufacturers of conical implant-abutment connections (n = 7 per group) with 1 µl S. enterica lipopolysaccharide, they were fully submerged in 5 ml of human blood. Three implants from each system also served as controls and were inoculated with 1 µl of phosphate-buffered saline (Harder, Quabius, et al., 2012). Samples were incubated at 37°C on a rotating platform for a period of 24 h, and 500 µl of blood was collected after 1, 8, and 24 hours and compared to control samples of blood without implants which were stimulated with different concentrations of the same LPS (Harder, Quabius, et al., 2012). Using real-time qPCR and immunoassay to analyze the samples for changes in gene or protein expression levels of TNF-α, TLR-9, nuclear factor kappa light chain enhancer of activated B cells, IL-1β, IL-6, interferon-γ, they were able to match LPS induced stimulation of gene expression similar to all concentrations of the control blood samples in five implants from system one and three implants from system two (Harder, Quabius, et al., 2012). They concluded that although bacterial leakage is reduced in tapered implant-abutment systems, it does not prevent the leakage of endotoxins to the peri-implant tissue (Harder, Quabius, et al., 2012).

2.6 Chlorhexidine (CHX)

Considered one of the most widely used and safest oral antiseptic agents (Varoni et al., 2012); CHX is a bisbiguanide base, broad spectrum antimicrobial agent with both bacteriostatic and bactericidal properties; it is active against Gram-positive and Gram-negative bacteria as well as fungi and yeasts. With its cationic structure, it
attacks and binds to the negatively charged bacterial cell wall, bringing the osmotic balance out of sync (Toljanic et al., 1992; Varoni et al., 2012).

Early studies on chlorhexidine's ability to achieve plaque inhibition proved its superiority regardless of how it was applied, as a rinse or topical application (Jenkins et al., 1988). At high concentrations it has immediate bactericidal properties by causing cytoplasmic coagulation or precipitation (Jenkins et al., 1988; Puig Silla et al., 2008). Some attributed its prolonged bacteriostatic action to its adsorption to the pellicle (Jenkins et al., 1988). Others believe that its substantivity, the ability of a substance to bind to a contacted structure, which in the case of CHX refers to the glycoproteins that coat the oral mucosa and teeth and release active agents over a prolonged period is what mainly contributes to chlorhexidine's biofilm-inhibiting properties (Toljanic et al., 1992). More recently it has been shown to act bacteriostatically at low concentrations by helping release potassium and phosphorus out of the cell, inducing an osmotic destabilization (Puig Silla et al., 2008).

While it is considered one of the safest antiseptic agents in use, some side effects as a result of prolonged or inappropriate use have been reported. Tooth and mucosal discoloration, increased supragingival calculus, temporary dysgeusia, temporary burning and dry mucosa sensation, as well as rare cases of desquamative oral lesions and hypersensitivity have been reported in past studies, with all these being reversible once the treatment has been discontinued (Varoni et al., 2012).

2.6.1 CHX & Implant Decontamination

Groenendijk et al. (2004) can be considered one the first to look at decontaminating the IAI with the use of chlorhexidine. In their double-blind split-
mouth design a 0.2% CHX solution was used in twelve edentulous patients who were
to receive four mandibular implants to support an overdenture (Groenendijk et al.,
2004). The experiment started at second-stage surgery (week 0); to have bacterial
baseline numbers, samples were collected immediately after removal of the cover
screws using sterile paper points. Immediately after sampling, inner parts of the
implants were rinsed with physiological saline solution and in the control group,
abutments were torqued into place with a force of 20 N as recommended by the
manufacturer (Groenendijk et al., 2004). For the test group, the internal parts of the
implants were dried using sterile paper points followed by injection of 0.2% CHX
solution and fixation of the abutment (Groenendijk et al., 2004); prosthetic treatment
started two weeks thereafter. Six weeks from the start of the study, abutments were
removed and once again using sterile paper points, samples were collected from the
fluids collected in the internal cavity of the implant (Groenendijk et al., 2004). Viable
bacteria were detected at baseline in 21 of the 46 implants with similar amounts of
CFUs in both groups; however at six weeks, 46% of the control implants and only
17% of the test implants had a total CFU of $\geq 10^4$ with the difference between the two
groups reaching statistical significance (Groenendijk et al., 2004).

Paolantonio et al. (2008) attempted for the first time to use CHX gel for the
decontamination of the internal implant cavity over a six-month period. In their
randomized clinical trial (RCT), baseline microbial samples were collected from all
implants ($n = 30$) by means of healing abutment removal and sterile paper points three
months after prosthetic restoration of all implants (Paolantonio et al., 2008). Prior to
abutment and crown placement, test implants received 1% CHX gel in the internal
cavity of the implant; microbial sample collection was repeated in the same manner six months later (Paolantonio et al., 2008). After six months, the total CFU in the test group was statistically significantly lower when compared to the control samples; while CFUs were the thirty to forty thousand mark for both groups at baseline, after six months the CFUs had more than doubled in the control group and dropped to a tenth of the baseline measured in the test samples (Paolantonio et al., 2008). PCR analysis of the samples did not result in significant differences between the two groups in regard to the frequency of monitored bacterial detection; the detection of specific bacterial was rather sporadic (Paolantonio et al., 2008).

In an attempt to assess the influence of CHX decontamination on host response parameters, D’Ercole et al. (2009) carried out experiments following similar methods as Paolantonio et al. (2008) in terms of timing of inclusion and baseline sample collection. However second samples were taken three months after completion of prosthetic treatment. Additionally in both instances, peri-implant crevicular fluid was collected via paper points to assess the activity of AST (D'Ercole et al., 2009), a cytoplasmic enzyme used to detect extent of cell death and magnitude of tissue destruction. PCR testing was also carried out to detect the frequency of occurrence of A. actinomycetemcomitans, T. forsythia, C. rectus, E. corrodens, F. nucleatum, P. gingivalis, P. intermedia, and T. denticola (D'Ercole et al., 2009). While significantly lower bacterial counts were detected in the test group after three months; no significant differences were detected in regard to bacterial frequency (D'Ercole et al., 2009). AST levels after three months remained similar to baseline levels, however a significant difference from 0.27 ± 0.12 to 0.41 ± 0.20 in the control group was
recorded, indicative of the reduced tissue destruction when 1% CHX gel is used (D'Ercole et al., 2009).
3. AIM OF THE STUDY

The objective of this prospective randomized clinical trial was to evaluate the biofilm reduction potential of 1% CHX gel when utilized in the internal cavity (screw hole) of Straumann Bone Level, NobelReplace CC or NobelActive implants at the time of placement. The implants were placed by experienced periodontists at two affiliated private clinics as well as by senior Graduate Periodontics residents at the University of British Columbia.

The following *hypotheses* were tested:

I. The use of 1% CHX gel reduces the formation of a biofilm in the internal cavity of the implant when utilized at the time of implant surgery and placement of a transmucosal healing abutment.

II. No statistical difference of biofilm formation or reduction should be noted between the two different implant systems.

III. Mainly anaerobic bacterial species should be detected in the implant screw hole.
4. MATERIALS & METHODS

Thirty patients (Male = 14, Female 16) with a total of forty-three implants (n = 43) were recruited to participate in this randomized clinical trial. The study population was recruited from patients who attended the Graduate Periodontics Clinic at the University of British Columbia (UBC), for placement of dental implants as part of their overall treatment plan as well as from two UBC affiliated private specialty clinics (Vancouver and Calgary). Human ethics approval was attained from the Clinical Research Ethics Board (CREB), UBC Office of Research Services. Prior to the surgical placement of the implant(s), the aim of the study was explained to the patients by the principal investigator or his representatives; all patients gave written consent in accordance with CREB requirements.

4.1 Patient Selection

Only patients who were to receive a NobelActive, NobelReplace Conical Connection internal connection (Nobel Biocare; Kloten, Switzerland), or Straumann Bone Level (Straumann; Basel, Switzerland) implant with a one-stage approach were recruited to participate in the study. The inclusion and exclusion criterion are listed in Table 1 & 2 below respectively.

Table 1. Inclusion criteria

<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Patients who had one or more NobelActive, NobelReplace CC, Straumann Bone Level implants</td>
</tr>
<tr>
<td>placed at the UBC graduate periodontics clinic or at two affiliated private specialty</td>
</tr>
<tr>
<td>clinics</td>
</tr>
<tr>
<td>• Patients requiring a one-stage surgical approach with the placement of healing abutments</td>
</tr>
<tr>
<td>immediately after surgical placement</td>
</tr>
<tr>
<td>• Partially edentulous</td>
</tr>
</tbody>
</table>
Table 2. Exclusion criteria

<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Allergic to chlorhexidine gluconate</td>
</tr>
<tr>
<td>• Currently being treated for periodontitis</td>
</tr>
<tr>
<td>• Fully edentulous</td>
</tr>
<tr>
<td>• Have uncontrolled diabetes</td>
</tr>
<tr>
<td>• Current or past smokers</td>
</tr>
<tr>
<td>• Antibiotic use in the three months prior to surgical implant placement</td>
</tr>
<tr>
<td>• Pregnant or lactating mothers</td>
</tr>
<tr>
<td>• Unwilling and/or unable to provide fully informed consent</td>
</tr>
</tbody>
</table>

A total of nine implants were placed at the Vancouver office while seven were placed at the office in Calgary. All the remaining twenty-seven implants were placed at the UBC graduate periodontics clinic.

4.2 Surgical Procedure

Prior to the surgery, patients were instructed to rinse with 0.12% chlorhexidine solution for one minute as part of the standard surgical protocol. Upon placement of the implant with the required torque suitable for a one-stage approach, the surgeon randomly assigned the implant(s) to one of the four groups, either to one of the two test groups (ST: Straumann Test, n = 9; NT: Nobel Test, n = 11) or to one of the two control groups (SC: Straumann Control, n = 11; NC: Nobel Control, n = 9). Prior to placement of the healing abutment, all implant screw holes were rinsed with about 20 ml of sterile saline solution and dried using surgical suction while with the suction, preventing further contamination of the screw hole by saliva or blood. For the SC and NC groups, healing abutments were placed after this step on the implants and tightened using finger pressure as indicated by the manufacturers. The test groups ST
and NT received 1% CHX gel made at a local compounding pharmacy placed on the entire thread portion of the healing abutments; the abutments were screwed into place with the same torqueing protocol as in the control groups. A final diagnostic radiograph clearly showing the threads of the implant was taken as a reference for the bone level at the time of placement.

Patients were instructed to rinse twice a day with a 0.12% CHX solution for a period of two weeks post-surgery and refrain from brushing or flossing the surgical site for two weeks. Additionally, patients were prescribed systemic antibiotics (Amoxicillin) for a period of seven days; in cases of penicillin allergy, an alternative antibiotic was prescribed to the patients. Over-the-counter analgesics were to be taken as needed. Patients were seen for follow up appointments two weeks post treatment for assurance of an uneventful healing.

4.3 Sample Collection

Three months post surgical placement, at the time of osseointegration testing and prior to restorative treatment, samples were collected from implant screw holes. Implant sites were isolated with sterile gauze and cotton rolls and kept dry using suction to prevent contamination of the screw hole with saliva (Figure 3). After careful removal of the healing abutments, biofilm samples were collected from the internal cavity of the implant screw holes using a sterile fine microbrush in a circular rotating motion. Particular attention was paid at the time of collection to avoid contact between peri-implant tissue and the microbrush. Microbrush tips were inserted into glass tubes (Figure 4) containing 3 mm glass beads, 0.6 ml Brain Heart Infusion
(BHI) and Agar mix (Becton, Dickinson and Company, Sparks, USA) and cut with a pair of sterile scissors.

![Image](image1.png)  
Figure 3. Isolated implant screw hole after healing abutment removal  

![Image](image2.png)  
Figure 4. Glass tube containing glass beads, BHI & agar mix

Collected samples were stored in refrigerators and transferred to the microbiology lab of the Faculty of Dentistry at UBC within 24 hours for further processing. Overnight shipping was utilized for samples collected at the Calgary office. Follow-up radiographs were also taken from all implants at this time to determine the success of implant placement and levels of crestal bone.

### 4.4 Bacterial Culturing

Glass tubes containing samples were stored for 30 min at 37°C followed by 60 seconds of vortexing to allow for proper mixing and release of the biofilm from the microbrush into the solution. 100 µl of the sample solution was mixed with 900 µl of BHI and repeated five times through serial dilution. Subsequently three 20 µl drops from each one of the five dilutions were placed on one 5% sheep's blood agar plate. To serve a control, three additional drops of 20 µl from the original undiluted sample
solution were placed on the same agar plate and allowed to dry (Figure 5). The process was repeated a second time for each sample to obtain two identical agar plates. The samples were labeled with each patient's ID number, as well as the date of culture. One agar plate was incubated at 37°C (VWR International; Randor, USA) for a period of three days under aerobic conditions; a second plate was placed in an anaerobic pouch system, AnaeroGen (Oxoid; Basingstoke, England) and incubated at 37°C for seven days.

Figure 5. Serial dilution on 5% blood agar petri dish after drying
4.5 Bacterial Counting & Analysis

Each petri dish was removed from the incubator after three or seven days and digital images (Canon Rebel T1i; Tokyo, Japan) were taken for the purpose of bacterial counting utilizing the image processing software ImageJ 1.46r (National Institute of Health; Maryland, USA). Additionally all cultures were counted manually and directly from the petri dishes as a secondary control to eliminate any uncertainties due to the close proximity of some cultures. Bacterial counts were recorded for all dilution levels; however only colony forming unites (CFUs) for dilution levels that resulted in bacterial counts ranging between 5 and 50 colonies and were present in all three samples of the same dilution level were considered valid and were entered into a computer database (Microsoft Excel Mac 2011; Microsoft Corporation, Redmond, WA, USA) used for further statistical analysis.

One sample from a single CFU was taken for Gram staining (Protocol, Fisher Scientific Company; Kalamazoo, MI, USA) and further analyzed under the microscope to identify bacterial species by their shape (i.e. cocci, rods, etc…).

4.6 Statistical Analysis

Statistical analysis was completed using the OriginPro statistical program (OriginLab, version 9.0; Northampton, MA, USA). Univariate descriptive statistics were conducted for all bacterial growth comparisons within the groups as well as between groups by utilizing the Mann-Whitney U test. Gender distribution was presented as a percentage; differences between the test and control groups were compared using Fisher’s exact test. The time post-surgery until sample collection between the two study populations was compared using the Student t-test. Microbial parameters were presented as the standard deviation and median (25th; 75th percentile
± SD). The p-value of 0.001 was considered to be of statistical significance for all parameters; however a p-value of up to 0.05 was considered for comparisons were statistically significant differences could not be verified.
5. RESULTS

A total of 40 implants from three centres were available for analysis. Three samples from the initial 43 were lost during the study period; two patients never returned for final osseointegration testing and one sample was mislabeled. All implants healed uneventfully and radiographic assessments at the time of osseointegration testing confirmed bone remodeling to be within the acceptable range (Albrektsson and Zarb, 1993). The group distribution can be seen in Table 3 below.

Table 3. Study population and group distributions

<table>
<thead>
<tr>
<th>Group Names</th>
<th>Number of Implants (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straumann Test Group (ST)</td>
<td>9</td>
</tr>
<tr>
<td>Straumann Control Group (SC)</td>
<td>11</td>
</tr>
<tr>
<td>Nobel Test Group (NT)</td>
<td>11</td>
</tr>
<tr>
<td>Nobel Control Group (NC)</td>
<td>9</td>
</tr>
<tr>
<td>Lost Sample</td>
<td>1</td>
</tr>
<tr>
<td>Dropouts</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>43</strong></td>
</tr>
</tbody>
</table>

Samples were collected from the test population at 73 to 203 days after implant placement (mean = 110 days) and from the control population at 70 to 133 days (mean = 98 days). The large variation in sample collection times was caused by the unavailability of some patients to attend their appointments at the necessary study interval times. However, utilizing the student t-test, the difference between the two groups was found not to be statistically significant (p > 0.05).
Figure 6. Comparison of total number of days post surgery (prior to sample collection) (± SD, median) of the test and control population. □ = mean values.

5.1 Implant Distribution

Figure 7. Percentage of patients with one or more implants
The majority of patients had one implant placed (63%), while 22% received two implants and the remaining patients (15%) received three implants.

5.2 Anatomical Implant Location

![Pie chart showing implant distribution between maxilla and mandible in (n; percentile).]

The anatomical distribution of the implants between the maxillary (n = 19) and mandibular (n = 21) jaw was 47% and 53% respectively. The null hypothesis was not rejected with a probability of 0.75181 (p > 0.05) using a two tailed Fisher’s Exact Test, thus confirming the even anatomical distribution in both test and control samples.

5.3 Gender Distribution

Table 4. Gender distribution in the test and control groups of Straumann and Nobel implants.

<table>
<thead>
<tr>
<th></th>
<th>Male CHX</th>
<th>Female CHX</th>
<th>Male No CHX</th>
<th>Female No CHX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straumann</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Nobel</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Total (n)</td>
<td>9</td>
<td>11</td>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>
There was an even distribution of total number of implants in males (48%) and females (52%) of the test and the control populations. A one-tailed Fisher’s Exact Test was carried out to compare the gender distribution between the groups; the probability of the null hypothesis being true was 0.62441 ($p > 0.05$) and therefore there was no statistical difference between groups.

### 5.4 Different Bacterial Culturing Conditions

Univariate Mann-Whitney-U tests were used for the assessment of all CFU results. Statistical examination of the aerobic and anaerobic culturing methods for all the test groups (ST + NT) combined ($n = 20$) is shown in Figure 10 below and for all the control groups (SC + NT) combined ($n = 20$) is shown in Figure 11; there were no significant differences when looking at the CFUs/ml ($p > 0.05$). As a result of these
similarities and for ease of presentation, the majority of future comparisons as well as graphical presentations are shown for the aerobic culturing method.

Figure 10. Boxplots of total bacterial counts (CFUs/ml ± SD) of both Straumann & Nobel test groups (n = 20) comparing aerobic and anaerobic culturing methods. No statistical significance of difference between the two growth methods (p > 0.05).
Figure 11. Boxplots of total bacterial counts (CFUs/ml ± SD) of both Straumann & Nobel control groups (n = 20) comparing aerobic and anaerobic culturing methods. No statistical significance of difference between the two growth methods (p > 0.05).

The distribution of the samples in the control populations and in the anaerobic test group were more even with the median clearly visible; however significantly more samples yielded zero growth in the aerobic test groups, resulting in the overlapping of the median and the 25th percentile boarder.

5.5 Total Test & Control CFU Comparison

The results comparing of ST + NT (n = 20) to SC + NC (n = 20) for both aerobic and anaerobic culturing methods are shown in Figures 10 and 11 respectively. Both comparisons resulted in statistically significant differences (p < 0.001), favoring the test groups with lower total CFUs/ml and clearly indicating the reduced levels of bacteria present in the CHX population. As previously reported, the median is not visible in the CHX population under aerobic conditions since the results more
frequently demonstrated zero growth in the samples. Overall a more even growth distribution was evident in the anaerobic population; however the results were not statistically significant.

Figure 12. Aerobic boxplots of total bacterial counts (CFUs/ml ± SD) of both Straumann & Nobel test groups (ST + NT; n = 20) compared to the total CFUs of both control groups (SC + NC; n = 20). Statistically significant differences between the two populations were recorded (p < 0.001).
Figure 13. Anaerobic boxplots of total bacterial counts (CFUs/ml ± SD) of both Straumann & Nobel test groups (ST + NT; n = 20) compared to the total CFUs of both control groups (SC + NC; n = 20). Statistically significant differences between the two populations were recorded (p < 0.001).

5.6 Straumann CFU Results

While the test population showed a median growth of zero (0 – 195,000 CFUs), the growth was significantly higher in the control population with a median growth of 95,000 CFUs (0 – 226,650,000 CFUs). Non-parametric analysis of the two samples showed a significantly lower growth potential in the test population (p < 0.001).
Figure 14. Aerobic boxplots comparison of total bacterial counts (CFUs/ml ± SD) of Straumann tests (ST; n = 9) to Straumann controls (SC; n = 11). Significantly lower CFUs in the ST population ($p < 0.01$).

5.7 Nobel CFU Results

Similar results to the Straumann implants were found to be true for the NT population with significantly lower CFUs in the test population ($p < 0.001$). While large variations between samples were present with a median of zero for NT (0 – 8,666,667 CFUs), the control group showed even higher growth potential with a median of 26,666,666 CFUs and a substantially higher maximum (41,667 – 500,000,000 CFUs).
Figure 15. Aerobic boxplots comparison of total bacterial counts (CFUs/ml) of Nobel tests (NT; n = 11) to Nobel controls (NC; n = 9). Significantly lower CFUs in the NT population ($p < 0.001$).

### 5.8 Straumann Test vs. Nobel Test CFU Comparison

To compare the growth potential which can also be considered the leakage potential between the two different implant systems, further comparisons were carried out looking at the total CFU differences between Straumann and Nobel implants in both the CHX population as well as the control group. Nobel implants showed a greater leakage potential with higher values for CFUs; however the difference between the two systems did not reach statistically significant rates ($p > 0.05$). CHX gel showed a similar bacterial reduction potential in both systems.
Figure 16. Aerobic boxplots comparison of total bacterial counts (CFUs/ml ± SD) of Straumann tests (ST; n = 9) to Nobel test (NT; n = 11). No significant difference between the two implant systems (p > 0.05).

5.9 Straumann Control vs. Nobel Control CFU Comparison

While the test groups showed comparable reduction results with no statistical difference at p > 0.05, control groups differed from one another. As previously demonstrated, both implant systems demonstrated greater growth potential when no CHX was used; however the Nobel implant system appears to have a greater bacterial leakage potential as a result of higher growth rates (p < 0.05).
Figure 17. Aerobic boxplots comparison of total bacterial counts (CFUs/ml ± SD) of Straumann controls (SC; n = 11) to Nobel controls (NC; n = 9). Significantly higher CFUs in the NC population ($p < 0.05$).

5.10 Implant Distribution by Total CFUs

Looking at the total number of implants in this study (n = 40), the figures above clearly demonstrate the growth-inhibiting potential of CHX with only thirteen test implants having growth levels < 1000 CFUs compared to only one to two implants in the control population. When looking at the extreme growth factors, the opposite was evident with nine control implants showing CFUs of > 10,000,000 compared to only one test implant.
Figure 18. Implant distribution based on CFU/ml and screw hole treatment (aerobic).

Figure 19. Implant distribution based on CFU/ml and screw hole treatment (anaerobic).
5.11 CFU Distribution Representation

Using digital images and manual counting to calculate the total CFUs, the CHX group clearly demonstrated a significantly lower growth potential ($p < 0.001$). Figure 20 shows the sample distribution based on CFU counts with thirteen implants in the test group showing no growth and lower overall counts for the remaining twenty implants.

![Figure 20. Average CFU formation on agar plates, (left) test sample, (right) control sample](image-url)
Figure 21. Aerobic sample distribution based on total CFUs.

5.12 Microscopic Analysis

Figure 22. Microscopic images of samples, species of the rod and cocci family are visible.

Microscopic examination of 15 implant samples from various populations showed the majority of the bacteria cultured to be of the Gram-positive coccoid species, only one sample had significantly higher Gram-negative bacteria. While
fourteen samples showed exclusively coccoid species, one sample showed populations of rod-shaped bacteria with coccoid species. Both variations were observed in samples collected from control groups.
6. DISCUSSION

Rehabilitation of missing teeth with dental implants has evolved significantly since the first implants were introduced; the same holds true for their success rates. Constant improvements to implant design and implant surfaces have led to shorter treatment times, decreased crestal bone remodeling as well as increased soft tissue health (Finne et al., 2007). Further improvements can certainly be achieved; nevertheless they are only attainable with novel ideas and never before considered aspects.

To the best of our knowledge, the prevention of biofilm formation in the implant screw hole with the help of antibacterial substances has never been studied. Only two studies known to date have looked at utilizing 1% CHX gel in the implant screw hole and these studies reported a substantial CFU reduction (Paolantonio et al., 2008; D'Ercole et al., 2009). Both these studies attempted however, to reduce the bacterial load once the biofilm had already formed. The composition of the biofilm makes it more resistant to host defense mechanisms as well as chemical treatments due to their decreased penetration into the biofilm structure (Socransky and Haffajee, 2002; Costerton et al., 2005). The longer a biofilm matures, the harder it is to eliminate with disinfecting agents such as CHX. A recently published study demonstrated the ability of 2% CHX to kill 20-100% of biofilm bacteria if the biofilm was not older than two weeks; however CHX’s efficacy decreased to only 10-30% of the biofilm bacteria once the biofilm had matured for three weeks or more (Stojicic et al., 2013). This biofilm property signifies the importance of preventing biofilm formation, especially in the threaded environment of an implant crew hole.
6.1 Study Timeline & Sample Processing

The mean sample collection time for the test population was 110 days and 98 days for the control population. The study aimed to set the timeline to 90 days post-surgery since most implant companies recommend a healing time of three months prior to restoration of the implants under standard conditions. This time should also allow for sufficient bacterial growth or prevention since baseline measurements taken in previous studies at the three-month mark demonstrated a significant presence of bacterial colonies in the screw hole (Paolantonio et al., 2008; D'Ercole et al., 2009).

Prior to sample collection, test culturing was conducted on multiple samples collected from patients at the University of British Columbia to assess the culturing protocol. Each sample was processed 10 times to yield ten samples out of the initial sample; half of these were stored at room temperature and half were refrigerated. On five consecutive days, one sample from each group was used and cultured both aerobically and anaerobically to simulate sample transfer delays and their effect on total bacterial growth; no differences were recorded for any of the samples up to the five-day limit. These findings confirmed two important aspects, the first being that the medium in the collection tubes did not promote further bacterial growth over time and second, even after storing the samples for five days, bacteria can still be cultured with the same accuracy as bacteria cultured on the day of sample collection.

Regardless of the pre-experimental findings, all possible measures were taken to allow for processing of the samples in the lab within 48 hours of collection; however on two occasions, samples were cultured six-days post-collection. Both samples belonged to the control population and were received late as a result of mailing delays
from the two affiliated offices. While one sample had 36,666,666 CFUs/ml, a number similar to other control samples, the second sample unexpectedly yielded no growth. It was the only implant in the control group with absolutely zero growth and the shortest period of time between surgery and sample collection (70 days). The combination of the delayed culturing as well early sample collection could have resulted in the zero bacterial growth. As this study involved multiple centres as well as a number of surgeons and staff, the possibility that the patient was labeled wrongly as being part of another group cannot be ruled out; however other variables such as tightening torque, patient hygiene, or even host response should also be considered as influencing factors.

6.2 Lack of Baseline Sampling

As part of our experimental protocol, no baseline samples were collected from the implant screw holes. While the three previous studies (Groenendijk et al., 2004; Paolantonio et al., 2008; D'Ercole et al., 2009) all recorded baseline samples, based on the sample collection time in our study such recording was considered irrelevant for two reasons. The first was that the purpose of our study was to assess biofilm formation or prevention and not immediate saliva contamination and the second was that the implants were considered to be sterile or free of a biofilm at the time of placement. Although the absence of a biofilm was never clinically tested, SEM images taken from new and freshly opened sterile healing abutments confirmed the absence of such microorganisms as shown in the images below (Figure 23 & 24). Higher magnifications revealed an increase in surface roughness; however this was
due to manufacturing processes and considered to be part of the desired surface texture by the manufacturer.

Nevertheless, even if the absence of bacteria could not be verified with 100% certainty for every implant used in our study, the fact that over half of the test samples (13/20) presented zero growth after being \textit{in situ} (on average for 110 days), speaks for the effectiveness of the CHX gel.

![Image](image.png)

\textbf{Figure 23.} Low magnification SEM image of a sterile Nobel healing abutment.
Figure 24. Higher magnification SEM images of the thread section in figure 23 of a sterile Nobel healing abutment. No microorganisms can be detected.

In an unexpected finding by Groenendijk et al. (2004), 46% of the baseline samples from freshly uncovered implants after cover screw removal tested positive for viable bacteria and this further increased to 87% within six weeks of abutment connections (Groenendijk et al., 2004). Since all implants in this study underwent submerged healing with a cover screw, the bacteria present in the internal cavities of the initially contaminated implants can only have accessed the cavity as a result of contamination during the first stage of surgery, three months earlier. These findings further signify the need to prevent bacterial survival in the implant screw hole at the first stage of surgery irrespective of whether a treatment is a one or two-stage procedure.
6.3 Microbial Reduction

The results of the current experiment clearly demonstrate the effectiveness of 1% CHX gel in reducing or even completely preventing the formation of a biofilm in the implant screw hole over a period of three months or longer; these results are consistent with previous studies (Groenendijk et al., 2004; Paolantonio et al., 2008; D'Ercole et al., 2009). A few test samples demonstrated significantly higher growth potential (higher CFUs) and could be considered as outliers. On a patient level, these differences can be attributed to variation in each individual patient’s oral bacterial species and their host defense. Given the nature of clinical studies, patient variations could potentially result in differences; however, the bacterial growth reduction pattern was consistent with other test implants. In three patients, more than one implant was placed and at least one of the implants was allocated to a different group to better assess individual variations. In all patients, even when the test samples showed signs of substantial bacterial growth, the control implants showed an even higher growth potential (Table 5).

Table 5. Individual bacterial growth variation, similarly shaded areas represent same individual.

<table>
<thead>
<tr>
<th>Implant ID</th>
<th>Site</th>
<th>Group Allocation</th>
<th>CFUs/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>15</td>
<td>Test</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>16</td>
<td>Test</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>25</td>
<td>Control</td>
<td>17,167</td>
</tr>
<tr>
<td>24</td>
<td>46</td>
<td>Test</td>
<td>80,000</td>
</tr>
<tr>
<td>25</td>
<td>36</td>
<td>Control</td>
<td>6,833,333</td>
</tr>
<tr>
<td>32</td>
<td>16</td>
<td>Control</td>
<td>616,667</td>
</tr>
<tr>
<td>33</td>
<td>15</td>
<td>Control</td>
<td>683,333</td>
</tr>
<tr>
<td>34</td>
<td>14</td>
<td>Test</td>
<td>76,667</td>
</tr>
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</table>
While the results of our study are consistent with previous ones, the parameters assessed differ significantly. All previous publications have looked at bacterial reduction in the screw hole at the time of final abutment placement and samples were collected using sterile paper points. The flaw in these studies lies both in the timing as well as the sample collection method.

A few minutes after contamination of the implant cavity with saliva, the first stages of biofilm formation are initiated, namely the formation of pellicle followed by attachment of initial colonizers of the streptococcal species (Darveau et al., 1997; Lang et al., 2000); this process is similar in both natural teeth as well as implants (Lang and Berglundh, 2011). Once a biofilm is established, its physiological properties create resistance to chemical treatment. The only way to eliminate an established biofilm is to mechanically remove it; however the structure of an implant screw hole with its threads and small access hole make this process unpredictable if not impossible.

By utilizing sterile paper points in the implant screw hole for bacterial collection, only planktonic bacteria can be assessed since a biofilm would remain protected by threads. The species in a biofilm do not float freely in the internal cavity, but are attached to its walls where they continue to grow and produce endotoxins. By using fine-tipped sterile microbrushes, we were able to scrape the internal thread of the implant screw hole to investigate the reduction or prevention of such biofilms. Nevertheless, the CFU counts in this study are most likely an underestimation of the true levels of contamination in the implant screw hole, since complete biofilm removal with the microbrush and subsequent culturing is highly unlikely.
While the CFU results for the test groups did not differ significantly from one another, there were statistically significant differences between the Straumann and Nobel implant systems with less growth in the former if CHX gel was not applied. Since more than half of the implants in either system were placed by experienced periodontists, clinician inexperience is not likely a causative factor.

Both implant systems incorporate a platform-switching concept with a conical abutment connection. While this connection type has been shown in a number of publications to have the smallest microgap measured to date, lack of such a microgap has never been reported in a screw-retained abutment system (Ricomini Filho et al., 2010). No manufacturer specifications for the microgap could be found for either implant system. Both companies, however, reference studies conducted by non-company researchers on final abutments and report the microgap dimensions to be less than 1 µm. Oral bacterial species have a diameter ranging anywhere from 0.1-1.5 µm (Jansen et al., 1997) while the microgap width has been measured by various methods to be anywhere from >1 µm under non-load baring to 22 µm under a force of 100 N (Rack et al., 2010). Misfits further increase when abutments of other manufacturers are used, with reports as high as 10.62 ± 2.16 µm under non-load bearing conditions (de Morais Alves da Cunha et al., 2012). Not only are these microgaps large enough for the passage of microbiota, but also their endotoxins, which are significantly smaller in size and induce inflammatory reactions in the host, can freely pass through and result in bone resorption at the implant-abutment interphase (Ozaki et al., 2009). This causality was confirmed in a study which looked at the distance of the microgap to the peri-implant tissue; it proved the presence of a
correlate between the distance and the amount of pro-inflammatory cytokines present in the peri-implant crevicular fluid (Boynueğri et al., 2012).

6.4 Microgap & Torque Force Relationship

The above microgap measurements were conducted under the recommended final abutment torque values of 25-35 Ncm depending on the implant system. However, healing abutments are hand-torqued into place, which translates into significantly smaller torque values and therefore increased microgaps. In a study by Hill et al. (2007), the authors measured the mean torque generated by general dentists in a limited access simulation model. Fifty-six volunteers were asked to apply maximum torque on an abutment screw three times with one-minute rests in between. The majority of the dentists were male (82%) and right-hand dominant (95%), with various levels of expertise and years of experience. They were grouped based on their years of dental experience, either over or under 20 years. The mean torque generated by the over 20 years of experience group was 13.5 Ncm while the less experienced group reached a mean value of 12.9 Ncm, not a statistically significant difference (Hill et al., 2007). Values as high as 36 Ncm have also been reported, but are not considered to be achievable by the majority of the population and these were not under similar space-limiting conditions (Goheen et al., 1994).

Similar differences were reported by Gross et al. (1999) when comparing manual torque drivers from five different implant systems and the clinicians’ abilities to apply maximum force without the help of a wrench. The nine operators were able to achieve torque values ranging from 9.4 to 19.9 Ncm; these values were considered 32-76% below the manufacturer’s recommended force (Gross et al., 1999). Aside
from the force achieved by fingers alone, there is also high variability in the perceived force obtained. Goheen et al. (1994) conducted a study in which experienced oral surgeons and prosthodontists were instructed to apply a torque of 10, 20 or 32 Ncm by only using a driver. The variation in the applied force ranged between 0.7-18.1 Ncm, 1.4-33.7 Ncm and 8.2-36.2 Ncm respectively for each of the indicated force values (Goheen et al., 1994).

While in the present study it appears that the Nobel implant system is inferior to the Straumann system when it comes to bacterial leakage and biofilm formation, similarities between the test groups demonstrate the positive effect that 1% CHX has on preventing biofilm formation regardless of any mechanical inferiorities or torque variations. The increased exposure time of the Nobel screw hole to the oral environment can potentially be one contributing factor to higher CFUs in the NC group when compared to SC. While the Straumann system has a pre-mounted transfer piece that may reduce saliva contamination during surgical placement, the Nobel implant screw hole is not isolated in the same manner. Whether these statistical shortcomings of the Nobel implants translate into clinical relevance is unknown and need to be investigated in future studies; however any bacterial contamination should always be considered a long-term risk factor and should be eliminated if possible, regardless of their clinically significant effects.

6.5 Bacterial Identification

Using PCR testing, Paolantonio et al (2008) and D’Ercole et al. (2009) attempted to identify bacterial species found in the internal cavity of implants. However, as their clinical findings were inconclusive, a specific identification was not
attempted in the current study. A large proportion of periodontal pathogens are considered to be Gram-negative anaerobic bacteria, some of which have also been determined to play a factor in the development and progression of peri-implantitis (Slots, 2010; Laine et al., 2013; Cortelli et al., 2013). Since no statistical difference between aerobic and anaerobic culturing was recorded, the bacterial population is believed to be either facultative anaerobe or aerobe.

Bacterial Gram staining was conducted on fifteen of the cultured samples to identity the frequency of occurrence of Gram-negative species. Surprisingly, 14/15 samples showed large populations of Gram-positive coccoid species. While experimental error cannot be ruled out, the majority of early colonizers belong to the streptococcal species such as *S. sanguinis* and *S. gordonii*, to name a few (Darveau et al., 1997; Aas et al., 2005). Also, recent studies have indicated that Gram-positive cocci have the highest frequency of detection in healthy implant sites (Shahabouee et al., 2012) which is consistent with our findings. All implants in the study were considered to be peri-implantitis free and radiographic evaluation did not show signs of significant bone loss beyond what is consistent with crestal bone remodeling (Albrektsson and Zarb, 1993). Early implant loss has been reported to occur in about 2.5% of all implants placed (Berglundh et al., 2002; Roos-Jansåker, Lindahl, H Renvert and S Renvert, 2006a), given that endotoxins from trapped bacteria in the implant screw hole have been shown to affect peri-implant tissue and induce a host immune response (Broggini et al., 2006; Harder et al., 2009). Decreasing this bacterial load could further increase short and long-term implant success.
Since peri-implantitis is an infectious disease, the treatment of the implant screw hole as well as its prosthetic components with CHX, or even the replacement of the abutments, should be considered a viable option to achieve the highest degree of treatment success due to elimination of the bacterial reservoir.

6.6 Internal Contamination

Patient compliance and oral hygiene (OH) habits, or oral health in general, can significantly influence the success of implant treatment. Poor OH leads to increased plaque accumulation and has been linked to crestal bone changes (Lang and Berglundh, 2011). Nevertheless, all patients in the current study had previously been screened for implant treatment and good levels of oral hygiene were considered a prerequisite for implant rehabilitation.

Two pathways for the contamination of the internal cavity of the implant system have been proposed as indicated in Figure 25. The implant system can be contaminated from bacteria traveling from the oral cavity into the microgap (inward growth) or bacteria can remain trapped inside the implant (outward growth) from the first surgical procedure (Aloise et al., 2010; Tripodi et al., 2012; Faria et al., 2011; Groenendijk et al., 2004), or a combination of both.
Figure 25. Contamination pathways of the internal implant cavity. m = microgap. Inward growth supplied by bacteria in saliva, outward growth as a result of contamination during placement.

Although patients included in the study were considered to have a high level of compliance and good oral hygiene habits, it is not possible to determine the source of contamination within the limits of this study. Results from the test population indicate that regardless of the source of contamination and OH effectiveness, application of 1% CHX gel significantly reduced bacterial inward growth or survival within the implant microgap as well as bacterial outward growth.

6.7 Limitations

Many shortcomings have already been discussion in previous sections. Due to the stringent inclusion and exclusion criteria and limited study period, recruitment of the study population was only possible with the help of multiple surgeons from three different centers. There are advantages to performing a multicenter study, such as reduced bias, and the results obtained by everyday clinicians rather than those from
certain highly qualified practitioners may be more reflective of clinical reality. Nevertheless, differing clinical experience levels may lead to increased risks of error including possible mislabeling or shipping delays of collected samples, differences in the amount and duration of irrigation performed at the time of implant placement, and differences in the amount of CHX gel applied to the screw hole. Future protocols should take all these factors into account and develop more reproducible methods of gel application and rinsing. Also a “learning curve” was experienced by most clinicians, as far as the study protocol was concerned.

Lack of standardization of the healing abutment’s shape and dimensions can further influence soft tissue attachment, bacterial penetration, possible contamination at the time of sample collection due to a narrower soft tissue collar and make SEM analysis difficult. Furthermore, standardization of the abutment torque values can further decrease variations in the microgap dimensions, while controlling for patient compliance by means of plaque measurements could explain some outlier values.

The short study period of three months is not sufficient to address concerns regarding CHX efficacy over long periods of time; however, past studies have demonstrated its effectiveness under load baring conditions even after a period of six months (Paolantonio et al., 2008).

6.8 Conclusion

Based on the results of this multicenter study, the use of 1% CHX gel at the time of placement can significantly reduce biofilm formation in the implant screw hole and maintain this effect for 90 days or longer (average 110 days); its use is strongly recommended at any stage in the rehabilitation of an edentulous site with an implant
as outlined in Table 6. The use of CHX can further reduce the risk of cross contamination of implants in patients with a history of periodontal disease.

Table 6. Recommended protocol for the use of 1% CHX gel in the implant screw hole.

<table>
<thead>
<tr>
<th>1% CHX to be used at time of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• First stage surgery, regardless of cover screw or healing abutment use</td>
</tr>
<tr>
<td>• Any abutment repositioning</td>
</tr>
<tr>
<td>• Peri-implantitis treatment</td>
</tr>
<tr>
<td>• Every exposure of the implant screw hole to the oral cavity</td>
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6.9 Future Direction

Although results from this study favour the use of CHX gel, examination of the effects of risk factors such as smoking, diabetes and OH should be considered with larger sample sizes as well as longer study durations. Furthermore, in order to better understand the bacterial pathway of internal contamination, analysis similar to this study should be conducted on implants after second stage surgery. Any detected pathogens in freshly exposed implants would be as a result of contamination during first stage surgery. Pyrosequencing can also be utilized to accurately identify the bacterial present.
REFERENCES


Hermann JS, Schoolfield JD, Schenk RK, Buser D, Cochran DL (2001). Influence of


