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Time of Exposure: A Major Factor in the Attachment of Bacteria

by

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Thesis submitted to the Faculty of the Department of Periodontics, Army Postgraduate Dental School, Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Master of Science 2020

THESIS APPROVAL SHEET

Time of Exposure: A Major Factor in the Attachment of Bacteria

This thesis is submitted by Dr. Anthony A. Vargas and has been examined and approved by an appointed committee of the faculty of the Uniformed Services University of the Health Sciences.

The signatures that appear below verify the fact that all required changes have been incorporated and that the thesis has received final approval with reference to content, form and accuracy of presentation.

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8 JUN 2020

ABSTRACT

Time of Exposure: A Major Factor in the Attachment of Bacteria.

CPT Anthony Vargas, DDS, MS Oral Biology, 2020

Thesis directed by: COL Kenneth Erley, DMD, Assistant Program Director, US Army Advance Education in Periodontics Program.

Purpose: The purpose of this study was to evaluate the ability of bacteria to attach to titanium at different durations of exposure. Furthermore, this study evaluated the difference in titanium binding capability between organisms, and also compared the ability of bacteria to bind to zirconia vs titanium. This study focused on bacteria which are associated with oral health, rather than known disease pathogens which were the focus of previous studies.

Methods: Bacteria for use in this study were selected by evaluating literature which describes species associated with oral health. Growth curves were then established for each bacteria species. Three different sets of trials were run: 1.) Titanium discs inoculated with *Streptococcus Oralis* and exposed for 1-, 3-, 5-, 8-, and 11-minute time intervals. 2.) Multiple species of bacteria [*Streptococcus mitis, Streptococcus Oralis, Gemella. hemolysans, Pseudomonas aeruginosa, Streptococcus gordonii, Granulicatella adiacens,* and *Neisseria flavescens*] were used to individually inoculate titanium discs for a three-

minute time duration. 3.) *S. oralis* was used to inoculate titanium and zirconia discs, OD was used to evaluate percent bound/ percent unbound bacteria.

Results: The results for the time interval study in percent bound were as follows: 1-minute – 10%, 3-minute – 21%, 5-minute - 26%, 8-minute – 67%,
11-minute – 93%. The comparison of bacteria at a three-minute time interval resulted in percentages of bacteria bound as follows: *N. flavescens* – 95%, *G. hemolysans – 93%, S. oralis – 38%, Streptococcus mitis - 35% G. adiacens - 32%, S. gordonii - 6%, P. aeruginosa - 5%.* The comparison of titanium and zirconia resulted in percentage bound as follows: titanium – 24% and zirconia – 34%.

Conclusion: Bacteria binding to titanium is time-dependent—the longer bacteria are exposed to titanium, the higher the attachment percentage. Bacteria binding to titanium is also species-dependent. This study showed dramatic differences in the ability of the selected species to bind to titanium at a 3-minute exposure duration. Bacteria associated with a healthy oral flora are capable of binding to titanium at short durations of exposure. However, there are significant differences in the attachment ability between organisms. No significant difference was identified in the attachment of bacteria to titanium compared to zirconia at a 3-minute exposure duration.

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CHAPTER 1: Introduction

Dental implants have become a reliable treatment option for the replacement of missing teeth. They have a high success rate, and are generally desired by patients. However, implants come with a set of their own problems. Ailing and failing dental implants are disappointing, adding additional stress to the patient and the provider. The most common problem that develops with dental implants is known as peri-implantitis. Peri-implantitis is a chronic disease process that develops around an implant which has integrated in the alveolar bone. As the disease progresses in severity and migrates apically, it can eventually lead to esthetic concerns and or loss of the implant.

Although less common than peri-implantitis, early implant failure, or failure to osseointegrate is another problem that occurs with dental implants. There are a number of theories as to why implants fail to integrate including overheating and infection/contamination during placement of the dental implant. The purpose of this study is to investigate the potential bacteria involved with implant contamination resulting in early implant failure.

Previous studies on this topic have focused on bacteria that are known pathogens in the process of periodontal disease. Initially, this may seem like a logical choice of focus because many of these pathogens have also been associated with the process of peri-implantitis. However, there are a number of reasons why bacteria associated with periodontal disease may not be the most clinically relevant bacteria to investigate. It is generally accepted that dental implant surgery requires a treatment planning sequence that treats existing bacterial infections present in the mouth before the implant surgery. This comes from the notion that active disease should be eliminated before an attempt to reconstruct or replace missing teeth. If a proper treatment planning sequence has been conducted, the amount of known disease pathogens associated with periodontitis would theoretically be minimal or nonexistent.

Despite proper treatment planning phases which eliminate disease and the use of sterile surgical equipment including sterile surgical fields, early implant failure still happens. This study investigates the potential of bacteria present in a healthy oral flora to bind to implant surface materials, which contribute to one of the possible mechanisms of early implant failure at the time of surgery. More specifically, this study investigates the ability of bacteria to bind to implant surfaces at short durations of time in comparison with most previous studies. Another unique feature is that this study utilizes bacteria which are commonly associated with oral health and evaluates their ability to bind to implant surfaces. This study also evaluates the ability of these bacteria to bind to titanium compared to zirconia surfaces.

Literature Review

There has been significant research established regarding the colonization of bacteria on titanium surfaces and dental implants. However, the vast majority of studies have focused on bacterial colonization in regards to peri-implantitis and long term bacterial biofilm development. These studies consisted of exposure durations from hours to days, or even weeks.[1,2] Although historically the emphasis has been placed on long inoculation times on dental implant materials, there have been select studies which focused on relatively short exposures. One study utilized titanium discs which were exposed for 30 to 120 minutes.[1] The study design involved a splint system which held titanium discs into the interproximal area of the patient’s teeth. The discs were then evaluated to determine the level of bacterial attachment. Although initial colonization data was obtained from this study, its main objective was to evaluate different surface topography of titanium discs. It found that smoother surfaces had less bacterial attachment than rougher surfaces.[1] Al-Ahmad et al. published another study involving the same splint system which was held in place for 3 to 5 days.[2]

Another study utilized single-cell force spectroscopy to analyze the initial interaction between *Staphylococcus aureous* cells and titanium surfaces in durations of 1 to 15 seconds.[3] Although this particular study was limited to a *S. aureous*, which is not commonly described as a member of the healthy oral flora, its findings are relevant in that they found an increase in bond strength proportional to the increase in time of exposure. Furthermore, it was the only study found in the literature review that utilized durations of exposure similar to the parameters evaluated in the current study.

Previous studies on this topic have focused on bacterial pathogens which are known for their role in periodontal disease. For example, *P. gingivalis* is commonly studied organism in regards to its ability to attach to implant surfaces. While the study of these bacteria is relevant in regards to the disease process of peri-implantitis, it is unlikely to be applicable to the mechanism of early implant failure. When implant surgery is performed on a periodontally healthy patient, contamination of the implant with periodontal pathogens is unlikely. Therefore, the focus of this study is placed on bacteria associated with a healthy oral cavity.

Studies have investigated the ability of various species of bacteria to bind to titanium surfaces. These have included bacteria associated with periodontal disease as well as species associated with disease commonly found outside of oral cavity. Although there have been previous studies which included opportunistic pathogens such as *Pseudomonas aeruginosa*,[4] the literature review did not find studies that focused on the mainly gram positive bacteria associated with oral health. The current study is unique in that perspective. The study regarding *P. aeruginosa,* found that it was able to colonize titanium surfaces with roughness less than 0.5 nm, and that *P. aeruginosa* was not able to colonize surfaces with less than 1 nm in undulation.[4]

Titanium alloy powder has also been utilized to study binding characteristic of bacteria. This study tested three different bacteria species to evaluate the difference in binding characteristics of titanium powder compared to titanium powder coated in albumin. The results showed that the tested bacteria had different levels of attachment. When titanium was coated with albumin and saliva, the attachment of *Actinomyces vicosus* and *actinobacillus actinomycetemcomitans* were significantly decreased. *Porphyromonas gingivalis*, on the other hand, did not show a significant decrease in binding.[5]

A scanning electron microscopy study was utilized to evaluate the binding characteristic of *P. gingivalis, A. viscosus, and Streptococcus sanguis.* These bacteria were exposed to the titanium surfaces for 60 minutes. This study found that surface roughness affected the ability of these organisms to bind.[6] A similar study evaluated the effect of calcium, fibronectin, and albumin on *Streptococcus mutans* and *Fusobacterium nucleatum,* as well as other species listed previously.[7] The findings of these studies support the assertion that the binding characteristics of bacteria are complex and subject to a number of environmental factors.[1-10]

The notion of a characteristic oral flora associated with oral health has been documented in the literature.[11,12] Subjects in a state of oral health—defined by absence of periodontal disease and dental caries—have exhibited a distinct oral flora.[11-12] Furthermore, this microbial community does not include the presence of keystone periodontal pathogens such as *P. gingivalis*.[11] In addition to defining the bacteria present in health, one study investigated the presence of the bacteria in specific locations within the oral cavity such as the tongue, cheek, vestibule, and palate.[12] This research suggests bacterial species most likely to contact and contaminate titanium surfaces during implant surgery.

Marketing claims have recently been made regarding the benefits of zirconia compared to titanium in terms of biological effects. However, information on bacterial adherence to titanium compared to zirconia is currently limited. A bench top study that evaluated different implant surfaces found that the zirconia implant was out performed by most titanium samples, as well as the bovine enamel control sample.[1] However it is important to note that the texture of the surfaces was the primary factor under investigation. Therefore, it is possible that the bacterial adherence count was elevated due to the texture rather than the material of the test sample. Furthermore, clinical studies have evaluated bleeding on probing scores (BOP) of zirconia implants and abutments compared to titanium. The current evidence, while limited, shows no significant difference between zirconia and titanium. However, some studies do associate higher BOP scores with zirconia products.[13-16] An additional objective of the current study is to compare bacterial binding capability of zirconia to titanium under short time of exposure.

CHAPTER 2: Materials and Methods

The protocol for this study was developed in three steps. First, the literature was critically reviewed to evaluate the techniques previously utilized in the study of bacterial attachment. A method was developed from analyzing a number of previously utilized procedures while considering availability of materials and equipment. There are a number of methods which utilized titanium discs found in the literature.[1-4,6-10] Next, improvements were made upon previous methods. In most of the previous studies, the material sample was inoculated and/or rinsed in a way which failed to assure complete collection of the bound bacteria. The experimental design was improved by submerging the disc in media so that 100% of bound bacteria would be collected.

Species of interest were then identified by selecting from available literature defining health-associated oral bacteria. This is in contrast to previous studies which evaluated bacteria associated with disease. Specifically, bacteria known to be a component of the normal oral flora were selected based on frequency of location, which was available in published studies (Figure 1).[12]

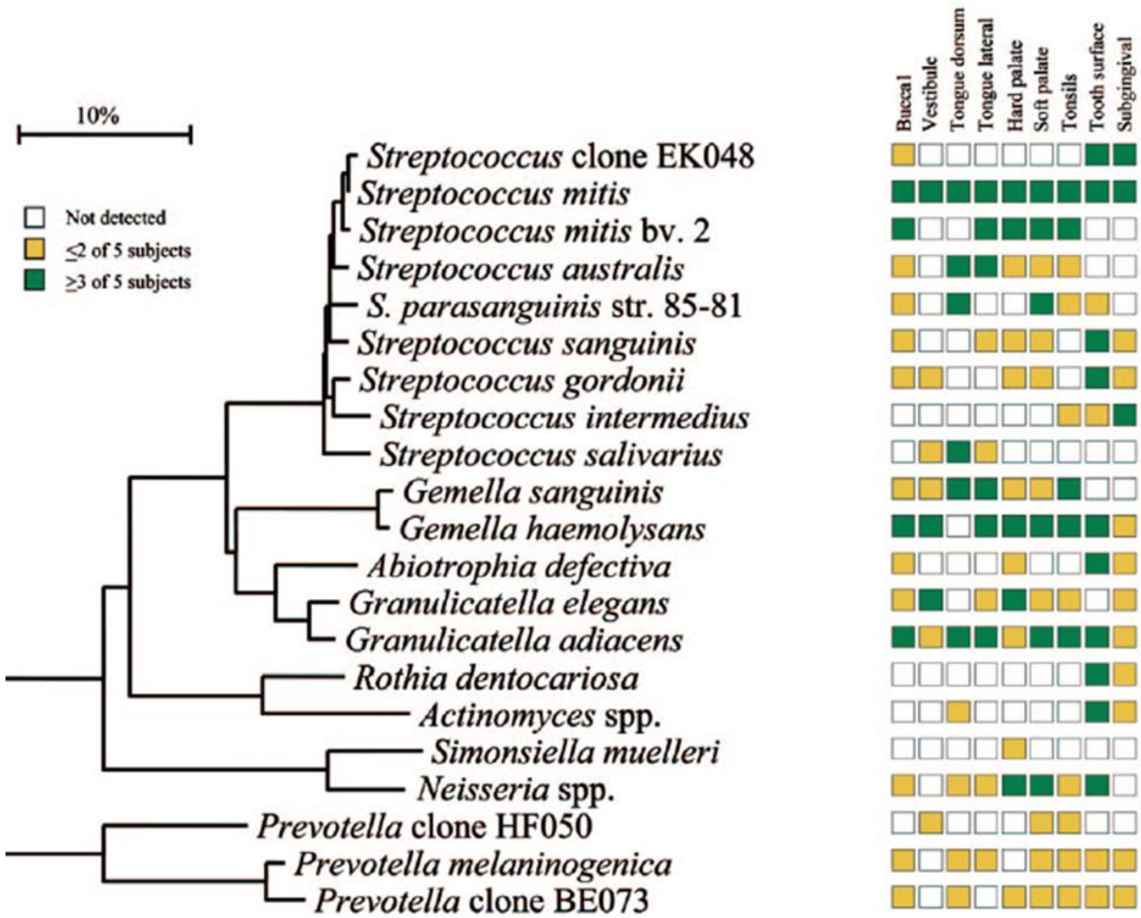

Figure 1. *Defining the Normal Bacterial Flora of the Oral Cavity.*[12] The diagram lists the bacteria that are considered to be components of the normal oral flora. Each column is labeled with a specific location in the oral cavity. The rows are labeled with individual bacteria. White boxes indicate that the bacteria was not detected. Yellow boxes indicate that the bacteria was detected in 2 or less subjects. Green boxes indicate that the bacteria was detected in 3 or more subjects.

The selected bacteria were also compared with those bacteria outlined in the results of another study regarding the healthy oral flora (Table 1).

Table 1. *Comparison of the Oral Bacterial Flora in Saliva from a Healthy Subject and Two Periodontitis Patients by Sequence Analysis of 16s rDNA Libraries.* Table consists of bacteria found to be associated with a healthy oral flora.[11]

Bacteria Associated with Normal Oral Flora
Actinobacillus minor
Gemella haemolysans
Haemophilus influenza
Haemophilus paraphropilus
Neisseria flavescens
Streptococcus mitis
Streptococcus oralis
Streptococcus sanguinis
Streptococcus pneumoniae
Streptococcus salivarius

This study established trends in bacterial flora components by comparing genetic sequencing analysis using 16s rDNA. Although the authors identified some species present in both healthy and periodontitis patients, the two flora were generally distinct.[11] Furthermore, the characteristic red complex bacteria[17] were not found in healthy patients in this study.

The final selection of bacteria was completed by cross-referencing the two studies, as well as considering availability of the species, equipment, and nutrition required for their growth. This led to the selection of the species utilized in this project (Table 2).

Table 2. *Bacteria selected for testing*. The bacteria selected are mainly associated with oral health. However, *S. Gordonii*, *P. aeruginosa*, and *N. flavescens* were also tested. These bacteria were commonly used in previous studies and serve as reference for the current study.

Bacteria	Characteristics
Streptococcus mitis	Gram +, facultative anaerobe, found frequently in healthy oral cavity, opportunistic pathogen, Alpha- hemolytic.
Streptococcus oralis	Gram +, facultative anaerobe, found frequently in healthy oral cavity, opportunistic pathogen, Alpha- hemolytic.
Gemella hemolysans	Gram +, facultative anaerobe, found frequently in healthy oral cavity, opportunistic pathogen.
Granulicatella adiacens	Gram +, facultative anaerobe, found frequently in healthy oral cavity, opportunistic pathogen, nutritionally deficient.
Streptococcus gordonii	Gram +, Facultative anaerob, known as an initial colonizer, forms attachment substratum for later colonizers on teeth.
Pseudomonas aeruginosa	Gram -, facultative anaerobe, opportunistic pathogen, not generally associated with healthy oral flora, used commonly in previous studies.
Neisseria flavescens	Gram -, aerobic found in normal healthy flora, but associated with meningitis, pneumonia, endocarditis and sepsis.

Bacterial growth curves were established by creating a 5-fold dilution series for each species using dilution media (20% BHI in phosphate buffered saline). Each dilution series was then distributed into two samples: one agar plate, and one well which was placed into the plate reader (Biotek Synergy 2). The two samples were incubated for 18 hours at 37^{o} C. Optical density was utilized to determine growth curves of the bacteria, and the time point at which the bacterial growth became exponential was noted. Colonies on the agar plates were then counted and correlated with T-max (time at which growth curve became exponential). Graphs were plotted to establish the relationship between optical density and colony count. Titanium discs were prepared by cutting 0.025mm thick 99.94% pure foil (Alfa Aeser #00360) using a hole puncher so that each disc had a uniform diameter of 7 mm. The discs were soaked in 100% ethanol for 3 minutes and then dried, and three experiments were prepared.

For the time of exposure test, the discs were inoculated for 1, 3, 5, 8 and 11 minutes with the *S. Oralis* bacteria dilution series. When the respective time durations were complete, the discs were rinsed and then placed and submerged into fresh media which was then incubated for 18 hours at 37^{o} C. For each respective dilution, the inoculum was also placed into fresh media and incubated for 18 hours at 37^{o} C. The plate reader was used to evaluate the growth over the 18 hour period of incubation. A chart was created showing the increase in optical density over time, which allows the changes to be observed relative to the original inoculation. These steps were completed for each dilution series.

The next test involved the comparison of the different bacteria species in their ability to bind to titanium. For this test, the dilution series for each bacteria (Table 2) were inoculated for a duration of three minutes. The discs were then rinsed and submerged into fresh media and incubated for 18 hours at 37^{o} C. For each dilution, a control was used to compare the original inoculum to the test sample (amount remaining on disc). A graph was created to visualize the changes over time.

The third test compared the differences in the ability of *S. oralis* to bind to zirconia and titanium. For each dilution series, titanium discs were inoculated with *S. oralis* for three minutes of exposure time. The discs were then rinsed and submerged into fresh media and incubated for 18 hours at 37 ° C. For each dilution, a control was used to compare the original inoculum to the test sample by placing the inoculum into fresh media in a separate well. Zirconia discs were then inoculated with *S. oralis* for an exposure time of 3 minutes. The discs were then rinsed and submerged into fresh media and incubated for 18 hours at 37 ° C. For each dilution, a control was used to compare the original inoculum to the test sample by placing the inoculum into fresh media in a separate well. Graphs were plotted to visualize changes over time. The growth curves created for the original dilution series were utilized to derive colony counts (CFU) from the optical density readings for all three tests.

CHAPTER 3: Results

Bacteria binding to titanium was found to be time dependent. As the amount of time the bacteria was allowed to be in contact with the titanium disc increased, the number of bacteria that remained bound to the disc also increased. Figure 2 displays the trend in optical density, which increased as time of exposure increased.

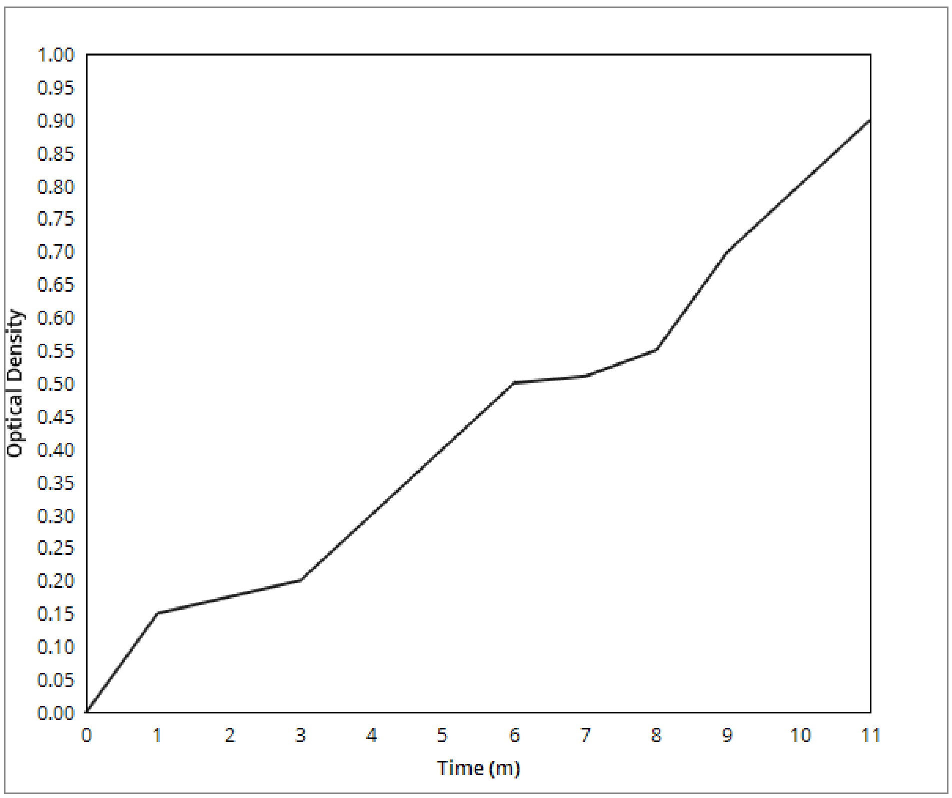

Figure 2. *Change in optical density over time*. As time of exposure of *S. oralis* to titanium discs increased, the optical density value from the sample increased.

The number of bacterial colonies present in the growth media increased as the time of exposure increased (Figure 3).

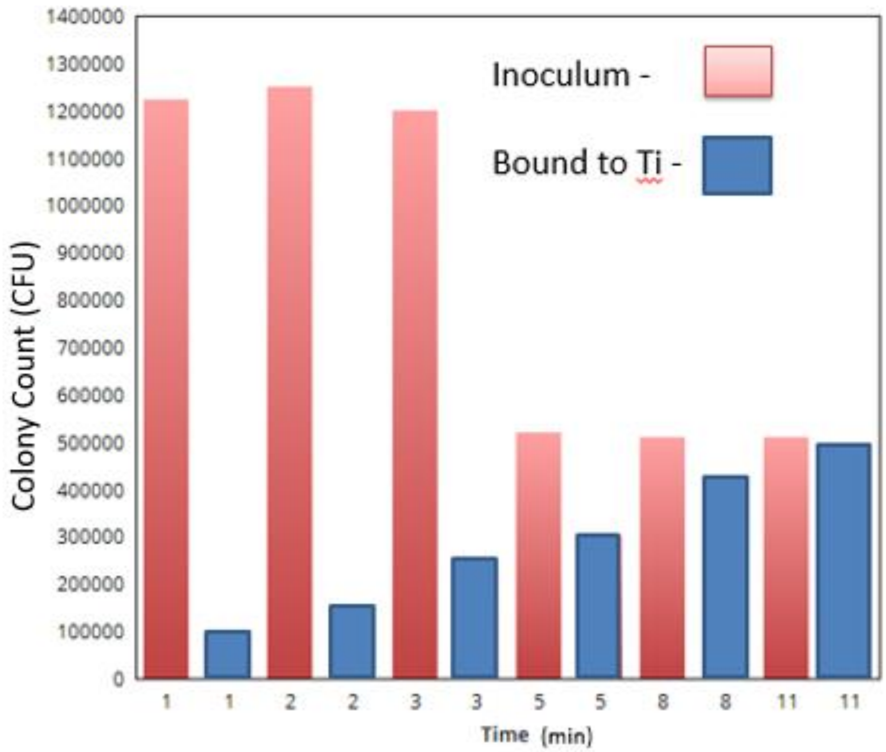

Figure 3. *Change in number of bacterial colony count over time*. As time of exposure of *S. oralis* to titanium discs increased, the number of bacterial colony formations increased. The red bar indicates total number of bacteria inoculated to disc. The blue bar indicates the total amount of bacteria that remained bound to the disc after its respective time interval. The X - axis represents the time of exposure in minutes. The Y- axis represents the colony count.

The percentage of bacteria bound to titanium discs increased as time of exposure increased (Figure 4).

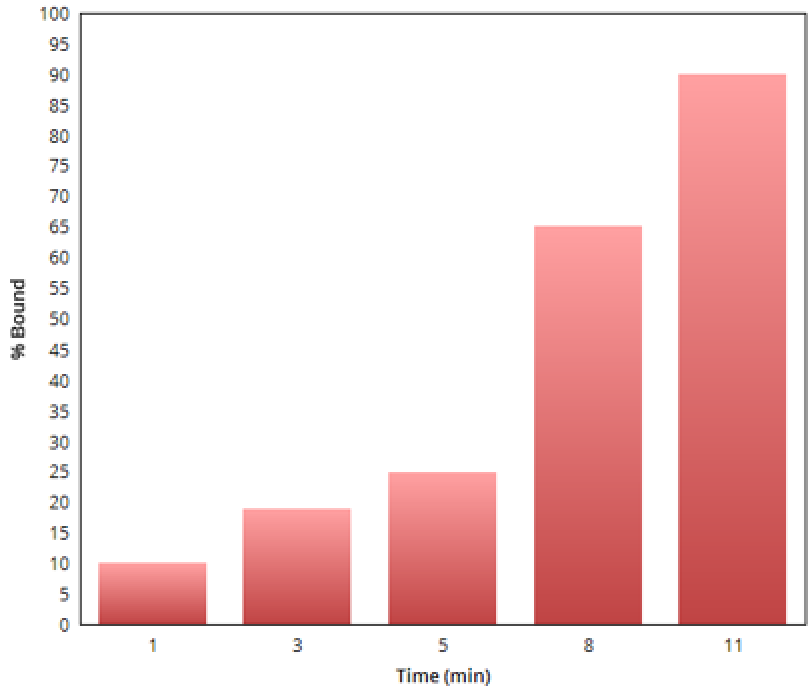

Figure 4. *The percentage of bound bacteria increased with exposure time*. The X – axis represents the time of exposure in minutes. The Y – axis represents the percentage of bacteria that remained attached to the disc.

The results showed that bacteria binding to titanium is species dependent. *N. flavescens* exhibited the highest percentage of bacteria that remained bound. The lowest percentage that remained bound was *P. aeruginosa*. The full spectrum of bacteria and their percentage that remained bound to titanium is presented in Figure 5.

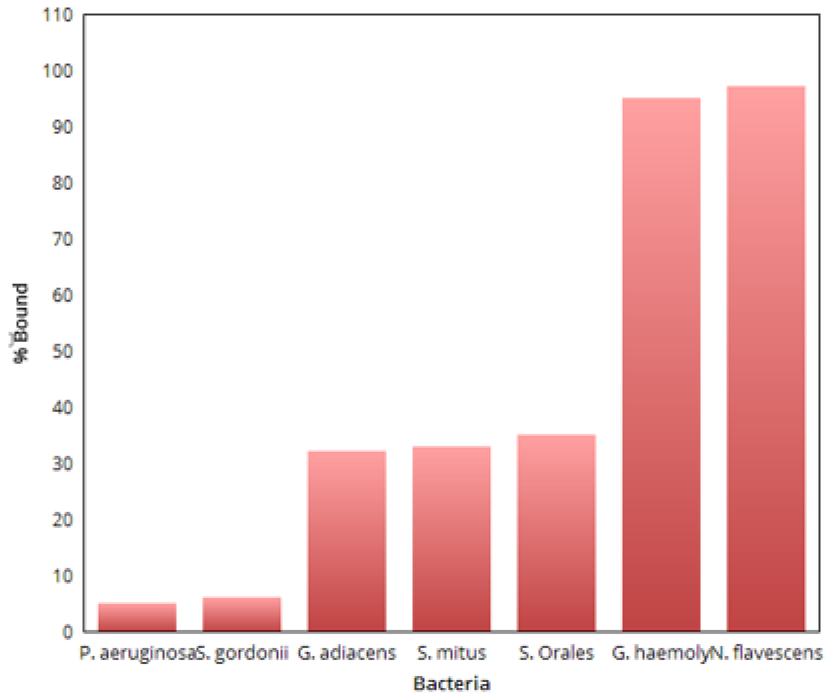

Figure 5. *Percentage of bound bacteria by species*. *N. flavescens* had the highest percentage of bacteria bound to the disc. *P. aeruginosa* had the lowest percentage that remained bound to the disc. *G. haemolysans* had a notably high percentage remain bound to the disc. *S. gordonii* had a notably low percentage remain bound to the disc.

N. Flavescens had the highest number of bacteria remain bound to the titanium disc. *P. aeruginosa* had the lowest number of bacteria remain bound to the titanium disc. *N. flavescens* had the highest number of bacteria present in its inoculation source. *S. Mitis* had the lowest number of bacteria present in its inoculation source. The number of bacteria that remain bound to the titanium disc, as well as the number bacteria (colony count) available in their respective inoculation sources is represented in Figure 6.

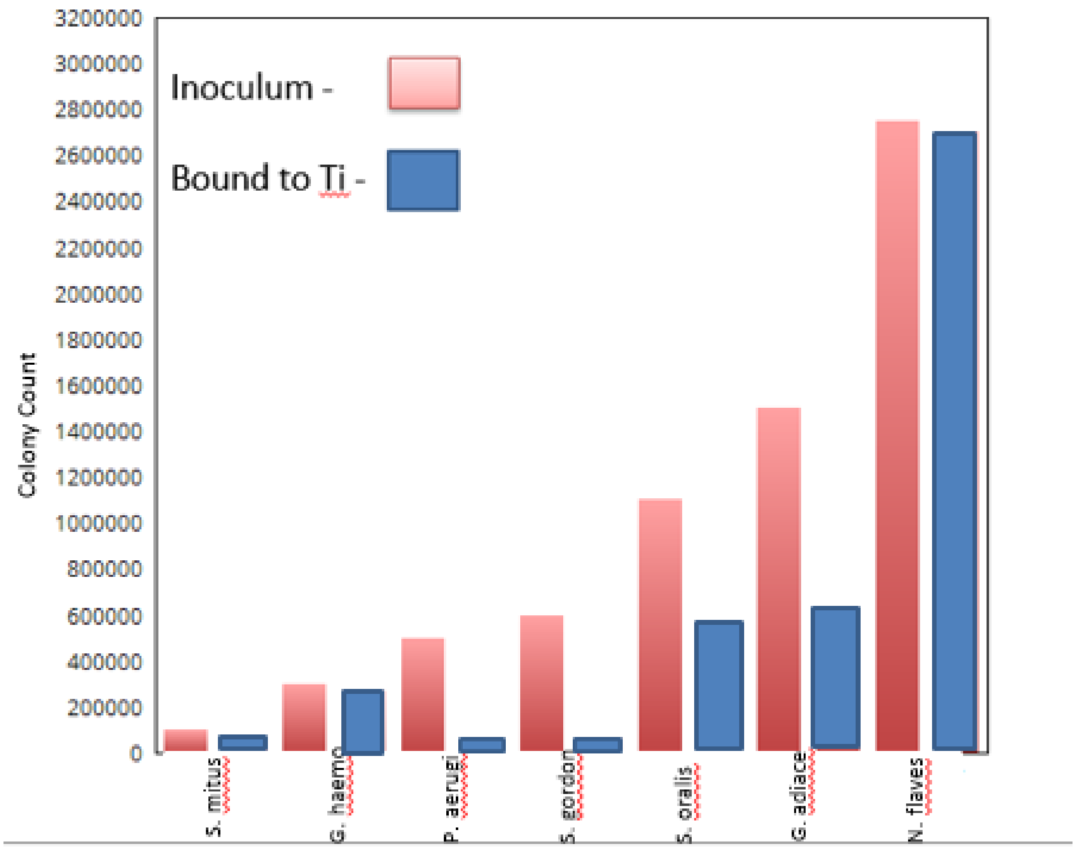

Figure 6. The number of bacteria that remained bound to the disc (CFU) compared to the number of bacteria that were present in their inoculation source. The red bar represents the number of bacteria available in the inoculation source. The blue bar represents the number of bacteria that remained bound to the disc. *N. flavescens* had the highest number of bacteria available in the inoculation source, as well as the highest number of bacteria that remained bound to the disc. *P. aeruginosa* had the lowest number of bacteria that remained bound to the disc. *S. mitus* had the lowest number of bacteria available in its inoculation source.

CHAPTER 4: Discussion

There are a number of factors that likely contributed to the observations in this study. Furthermore, these findings may have relevance in clinical implant surgery. Implant materials exposed to bacteria had an increased number of bacteria that remained bound to titanium as the time of exposure increased. Bacterial attachment is a complex process that involves many different factors.[1,2,7,10,17] Some species of bacteria require the formation of a biofilm or similar substance to be able to attach to specific surfaces. However, there are bacteria which do not require such prerequisites to initiate attachment. The idea that bacteria can attach to titanium without the formation of a biofilm was observed in this study. Nonetheless, biofilm should be considered a factor in the attachment of bacteria to titanium surfaces. It is possible that a specific species of bacteria shows a low affinity for titanium in a benchtop study, but displays a much higher ability to bind in a in vivo model where there are other factors such as saliva, and other contaminants at play. For these reasons, it is difficult to gain knowledge of the attachment characteristics of bacteria, but there is value in learning information about the ability of bacteria to bind to titanium in very isolated model that was used in this study.

Time is an important factor in the attachment of bacteria to titanium and the current study showed that there is an increase of bacteria attached to titanium as the time of exposure increases. While this in vitro study should not be used to directly guide clinical practice, the concept that longer exposure periods may increase bacterial attachment appears clinically relevant. Furthermore, there are additional factors in clinic settings that could increase the number of bacteria gaining attachment to the implant. For example, implants have different levels of surface roughness which could potentially increase the number of bacteria able to attach compared to the findings of this study. The current study used smooth titanium discs which generally collect less bacteria than titanium with rough surfaces.[1,10] The decision to utilize smooth discs in favor of roughened discs was made because the goal of the study was to evaluate the ability of bacteria to bind to titanium surfaces under conditions that are considered unfavorable for bacterial attachment, such as short exposure times, smooth discs, and bacteria which are associated with health rather than disease. Intuitively, increased surface roughness should increase bacterial attachment.

The current study is unique compared to most previous studies in that it focuses on testing bacteria that are associated with oral health, whereas previous studies tend to evaluate bacteria that are associated with periodontal disease.[1-9] While bacteria associated with disease likely play a role in peri-implantitis, disease-associated species may be less relevant to early implant failure in healthy individuals. Assuming that proper treatment planning phases are being followed, active periodontal disease would not be present during an implant surgery. Therefore, it is unlikely that periodontal disease pathogens are the causative agents for early implant failure when proper treatment planning phases are utilized.

Bacteria involved with periodontal disease may be capable of causing early implant failure, but that scenario is most likely to happen in clinical cases where proper treatment planning was not being followed. For example, a practitioner may complete implant surgery in a patient with untreated periodontal disease. It is recognized that there are cases where periodontal pathogens remain present despite the clinician’s best effort to provide proper treatment. However, the focus of this study is to investigate the potential of bacteria associated with oral heath to attach to implant surfaces.

Although the bacteria selected for use in this study are associated with oral health, it is important to note that any of these bacteria are capable of causing disease when an opportunity becomes available for overgrowth or the bacteria is transported into an environment where it dominates other bacterial species. The bacteria selected for the study were mainly gram-positive facultative anaerobes that are found in high number in the oral cavity.[11,12]

P. aeruginosa was also included, although it is not commonly associated with healthy oral flora.[12] *P. aeruginosa* was a common species used in previous studies regarding implant surfaces and bacterial attachment and was thus included for comparative value. *P. aeruginosa* is gram negative, aerobic, and rod shaped. It also has a single polar flagellum which allows it to achieve motility.[18] All strains of *Pseudomonas* contain endotoxin which is the main virulence factor of this bacterium. *P. aeruginosa* is known to cause various disease such as middle ear, corneal, and secondary burn infections. It has also been documented to cause meningitis and endocarditis following cardiac surgery.[18]

S. *mitis,* S. *oralis,* and *S. gordonii* are gram positive facultative anaerobes which are found frequently in the healthy oral cavity, throat, and nasopharynx. Although they are generally recognized as a component of the healthy oral flora, they have been documented to be the causative pathogen in some infective endocarditis cases, urinary tract infections, and other infections.[19,20] Furthermore, they are a component of the yellow complex bacteria and known as initial colonizers in the formation of microbial complexes in subgingival plaque.[17]

G. haemolysans is a gram positive and a facultative anaerobe. G. *haemolysans* is considered a component of the normal mucous membranes in the oral flora.[12,21] However, it has been reported to play a role in infective endocarditis in patients with comorbidities.[21] *G. adiacens* is also a gram positive, facultative anaerobe that is a component of the normal oral flora, genital, and intestinal tracts. Like many of the other bacteria in this study, it has been documented to cause sepsis, bacteremia and infective endocarditis. *G. adiacens* is considered nutritionally deficient and requires special supplementation to grow properly.[22]

N. flavescens is considered a normal component of the oral flora, as well as the upper respiratory tract and the oropharynx. It is a gram negative, aerobic diplococcus. *N. flavesscens* is rarely associated with disease, but special environmental conditions or immunocompromised patients can make an infection from *N. flavescens* possible. *N. flavescens* has been shown in the literature to be involved with infections of the blood, cerebrospinal fluid, and respiratory tract.[23]

There are a number of plausible explanations for the results obtained in this study. As discussed earlier, multiple factors influence attachment of bacteria to titanium surfaces, and although the findings of this study contribute a portion of the answer to these questions, it is recognized that there may be additional factors at play in live patients compared to a bench top study. For example, this study did not involve saliva, which contains many components which may influence the way bacteria bind to surfaces.[7,10,17] Furthermore, components of saliva can potentially alter the survivability of these species. The two bacteria which showed the highest binding to titanium in the study, *G. haemolysans* and *N. flavescens,* are not genetically related.[24] Therefore, it is likely that their mechanisms allowing rapid attachment to titanium are distinct. The details of the factors and mechanisms involved with the attachment of these two species would make for a worthwhile follow-up study.

Regarding time of exposure, the general trend observed in this study showed that increased exposure time resulted in greater bacterial attachment. Although some species of bacteria are capable of binding faster and in larger quantities than others, there is a certain amount of time that is required as a component of this process. Time allows for interactions between the cell and the surface to occur. The details of these processes are worth investigating in future studies, they are likely complex and may differ greatly between species and environmental conditions, as well as surface type and texture.

Recently there has been an increase in public demand for alternatives to titanium implants. This demand has likely risen from claims of biological compatibility issues, which have not been supported scientifically. Furthermore, arguments have been made that a white implant and or abutment is more esthetically pleasing than the metallic color of titanium, especially in cases that have experience gingival recession. Due to the increase in demand, main stream implant manufactures are now offering zirconia implant systems on the market. Despite claims of zirconia's advantages in biocompatibility, the results of studies comparing zirconia to titanium have shown a range from worse to no significant difference in BOP scores.[13-16] Currently, available evidence does not support the claimed advantages of zirconia in regards to biological compatibility as traditionally measured.[13-16]

The current study investigated unique aspects of bacterial binding characteristics compared to previous studies which focused on clinical gingival health. However, the results of this study follow the trend of previous investigations comparing zirconia to titanium. More bacteria were bound to zirconia than titanium, but no significant different between the two groups could be identified. At best, zirconia does not perform significantly better than titanium in most measurable aspects, but most evidence seems to point toward a slight underperformance. It appears that zirconia is a potentially successful alternative material for dental implants, but available evidence—including the present study—suggest disadvantages associated with this material.

When considering the reality of bacterial contamination, and the ability of bacteria to bind to titanium under short time durations, it is necessary to reassess the way antibiotics are utilized in implant therapy. Seventy-two percent to 74% of dentists routinely prescribe antibiotics prophylactically for implant placement.[25,26] The literature has described the advantages of prescribing prophylactic antibiotics for implant therapy as improved wound healing, reduced inflammation, and reduction in the rate of early implant failure. Clinicians who choose to utilize prophylactic antibiotics accept the risks of allergic reaction and promotion of antibiotic resistance.[27] Alternatively, choosing not to prescribe antibiotics eliminates the risk of allergic reaction, and promotes antibiotic stewardship within the profession. However, patient expectations may need to be managed as many patients expect to be given antibiotics when undergoing surgery.[27]

The American Dental Association (ADA) released an article stating that wide spread use of antibiotics is the driving force behind the public health problem of microbial resistance. Furthermore, the ADA stated that the only way to solve the problem is for practitioners to use antibiotics responsibly.[28] For example, antibiotics should only be used when there is an active infection, or when the patient has an additional risk factor. Based on these guidelines, it is difficult to make the argument that surgical implant placement should have antibiotic prophylaxis for healthy patients.

Perhaps the lack of evidence regarding prophylactic antibiotics for implant surgery has contributed to the tendency for dentists to perceive it as an insurance policy with little downside risk. Unfortunately, there are well documented risks of utilizing prophylactic antibiotics for the patient, community, and environment.[29] The overuse of antibiotics has a more profound effect than the issues surrounding healthcare. Excess antibiotic use also creates problems in public sewer systems and has become an overall environmental concern. Sewers and wastewater treatment plants collect waste which often contains mixtures of non-metabolized antibiotics, along with hospital waste and a plethora of bacteria. This creates an optimal scenario for the selection of antibiotic resistant genes.[30] Wastewater treatment plants allow the transfer of these genes to the freshwater ecosystem. The development of antibiotic resistance by microorganisms is relentless; despite national and international guidelines, little progress has been made on the efforts to reduce the use of unnecessary antibiotics.[30]

The results of the current study suggest that bacterial contamination can happen in times of exposure as little as one minute. When theoretically applying this knowledge to clinical practice it is obvious that an implant can easily be contaminated during surgery. There are often times when saliva, blood, soft tissue, or other contaminants come in contact with the implant, creating vulnerability to contamination of the implant surface. However, it is important to note that implant surgeries have a large range of accessibility and surgical difficulty. For example, maxillary implant surgery sites tend to be easier to isolate than posterior mandibular sites. It may be worth consideration to include contamination potential in the risk versus benefit analysis when considering prophylactic antibiotics. When contamination of the implant is anticipated due to patient related factors, it may be worth considering prophylactic antibiotics, especially when the patient already has conditions which may put them at a higher risk of infection. Ultimately, the clinician must use clinical judgement and carefully consider all possible risks and benefits when making a decision regarding antibiotic use for implant surgeries, as there are studies which support both sides of the argument.[27,31-34]

CHAPTER 5: Conclusion

Bacteria binding to titanium is time-dependent. The longer bacteria are exposed to titanium, the higher the percentage of attachment to titanium. While this study focused on a limited set of bacteria, it is likely that this conclusion can be applied to most bacterial species. If bacteria have the capability to bind to a surface, then increasing the exposure time will likely increase the number of bacteria that establish attachment. Bacteria binding to titanium is also species-dependent. This study showed dramatic differences in the ability of the selected species to bind to titanium at a 3-minute exposure duration. Of the bacteria tested in this study, *N. flavescens* had the highest percentage of bacteria bound to titanium at the 3-minute time interval. Multiple environmental, biological, and physical factors influence the ability of bacteria to bind surfaces.

Bacteria associated with a healthy oral flora are capable of binding to titanium at short durations of exposure. However, there are significant differences in the attachment ability between organisms. The duration of exposure utilized in this study is much shorter than previous studies. It showed that bacteria are capable of binding at these time intervals, and that some bacteria are capable of binding in high numbers despite highly restricted time intervals. There was no significant difference in the attachment of bacteria to titanium compared to zirconia at a 3-minute duration of exposure. This result is consistent with previous studies which compared titanium to zirconia and found that zirconia tended to perform similar or worse than titanium in the measured parameters. While the findings of this bench top study should not be used as a direct guide to clinical practice, the findings are useful in formulating an overall understanding of the capabilities of the bacteria that have a high potential to contaminate dental implant surgeries.

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