

A Comparison of Mechanical Tetracycline Disinfection of OSSEOTITE® and Nanotite® Implant Surfaces in a
Simulated Model of Peri-implantitis

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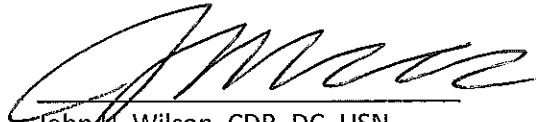
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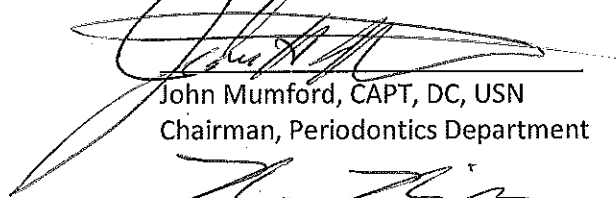
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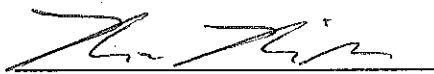
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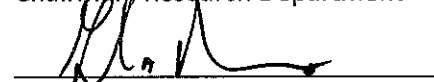
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ABSTRACT

Introduction: This pilot study will evaluate the efficacy of tetracycline in decontaminating OSSEOTITE and Nanotite implants within a simulated intrabony defect model. Implants will be secured in a sterilized resin model and contaminated with *S. Sanguinis* grown in a biofilm solution. Following incubation, tetracycline will be applied with cotton pellets in a standardized fashion, and residual bacteria will be put into solution and plated on blood agar plates. Final CFU counts will be tabulated allowing for a comparison of the ability to decontaminate OSSEOTITE versus Nanotite surfaces within a unique in vitro model. Positive and negative controls will be included for comparison. **Methods and Materials:** *S. sanguinis*, a gram-positive, facultative aerobe known to be an early colonizer of oral biofilms, will be incubated with sterile BIOMET 3i™ OSSEOTITE® and NanoTite® implants mounted in customized resin models that mimic a circumferential bony defect. A total of 18 implants--9 OSSEOTITE® and 9 Nanotite®--will be utilized and divided among control and treatment groups. For both types of implants, the following groups will be evaluated: treatment (n=5), positive controls (n=2) and negative controls (n=2). With the exception of negative controls, all implants will be incubated in a suspension (1.5×10^8 CFUs/ml) of *S. sanguinis* for 24 hours at 37°C. Negative controls will be incubated in sterile tryptic soy broth (TSB) culture for 24 hours at 37°C. Following incubation, each implant will be disinfected in a standardized manner using a technique described by Foisie (2011). For both treatment groups, sterile cotton pellets saturated with a tetracycline (50mg/mL) solution will be used to circumferentially wipe contaminated implant surfaces for 1 minute (20 wipes/min). Positive controls will follow the protocol for contamination with *S. sanguinis* but not undergo disinfection. Negative controls will also follow the contamination protocol, but will be disinfected using a sterile cotton pellet soaked in sterile saline. Immediately following surface disinfection, all implants will be removed from their models using sterile technique, and a sterile cover screw will be inserted into each implant before they are placed in phosphate buffered saline (PBS). After vortexing the implants to dislodge adherent bacteria, the remaining supernatant will be diluted, streaked onto blood agar plates and incubated. Following incubation, the plates

will be randomized, and colony forming units (CFUs) will be quantified by a blinded counter. A measure of the ability to detoxify the two implant surfaces will be obtained through statistical analysis of the residual CFUs from the treatment and control groups. Results: There are no results to report at this time. There is no funding anticipated for this project. **Discussion/Conclusion:** While there are no results from which conclusions can be made, the ability to decontaminate OSSEOTITE and Nanotite surfaces with mechanical tetracycline applications may differ due to the differences in surface topography of the implants. The simulated intrabony defect model incorporated into this experimental design heightens the validity of this in vitro study. If the results of this study show that increased surface area and complexity of implant surfaces harbor more live bacteria after mechanical disinfection, perhaps such increased surface complexities of dental implants enhance the risk for peri-implant mucositis and peri-implantitis.

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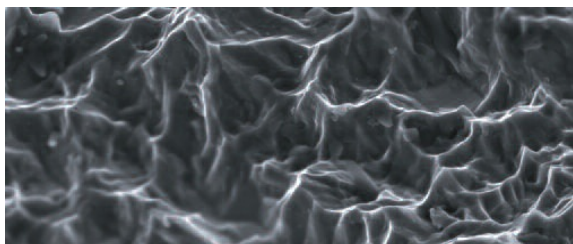
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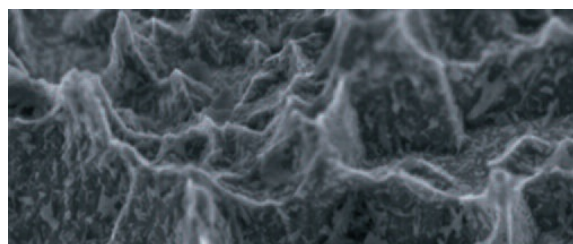
INTRODUCTION

This pilot study will evaluate the efficacy of tetracycline in decontaminating OSSEOTITE[®] and Nanotite[®] implants within a simulated intrabony defect model of peri-implantitis. Implants will be secured in a sterilized resin model and contaminated with *S. Sanguinis* grown in a biofilm solution. Following incubation, tetracycline will be applied with cotton pellets in a standardized fashion, and residual bacteria will be put into solution and plated on blood agar plates. Final Colony Forming Unit counts will be tabulated allowing for a comparison of the ability of mechanical tetracycline application to decontaminate OSSEOTITE[®] versus Nanotite[®] surfaces in a unique *in vitro* model. Positive and negative controls will be included for comparison.

Navy Dentistry principally utilizes the implant systems of two companies--Biomet 3i[™] and Nobel Biocare[™]. Within the BIOMET 3i[™] family of implants, the OSSEOTITE[®] and NanoTite[®] surfaces are most commonly used in clinical practice. OSSEOTITE[®] features a patented, acid-etched surface that enhances platelet activation and red blood cell agglomeration associated with implant attachment (Figure 1.). The newer NanoTite[®] surface maintains the purported benefits of the OSSEOTITE[®] surface with the addition of nanometer-scale calcium phosphate deposition resulting in a more complex surface topography and enhanced osseointegration. NanoTite[®] technology involves a patented process called Discrete Crystalline Deposition or DCD[™] that results in an increased micro-surface area of 200% for the NanoTite[®] surface compared to the original OSSEOTITE[®] implant (Suttin et al. 2006). While the increased surface complexity of the NanoTite[®] implant may enhance osseointegration and bone formation, decontamination of exposed implant threads in the case of peri-implantitis may prove to be a daunting task.



OSSEOTITE[®] Surface (20,000x)



NanoTite[®] Surface (20,000x)

Figure 1.

REVIEW OF THE LITERATURE

Peri-implantitis is induced by the same bacteria that cause periodontal disease (Mombelli et al. 1995), and tetracycline and citric acid are chemotherapeutic agents commonly used to treat tooth root surfaces contaminated by periodontal disease (Register and Burdick 1976, Lafferty et al. 1993, Terranova et al. 1986). Chlorhexidine is commonly given pre-operatively and post-surgically to reduce bacterial load and inhibit plaque formation. Loe and Schiott demonstrated that twice daily rinsing with 10mL of 0.2% chlorhexidine solution for 1 minute resulted in complete plaque elimination persisting for up to 22 days. The current study will evaluate the ability of tetracycline to detoxify the unique surfaces of OSSEOTITE® and NanoTite® implants after exposure to bacterial plaque. Peri-implantitis occurs in 16% of patients treated with implants after 9-14 years of function (Roos-Jansaker et al. 2007). The relevance of this study can be realized in its ability to provide options for dentists to treat peri-implantitis in two of the most commonly used implant systems. Failure to recognize and treat peri-implantitis early can result in devastating bone loss and possibly loss of the entire implant as seen in Figure 2.

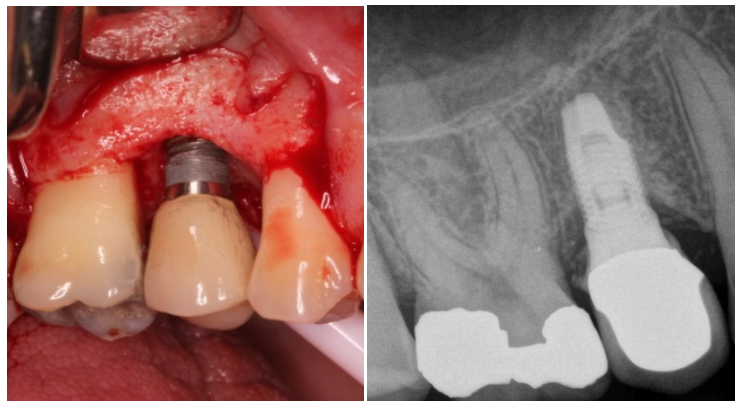


Figure 2.

Over the past 30 years, research has validated the success of osseointegrated implants as a viable alternative to fixed or removable prosthetic restorations (Iacono et al. 2000), and today implants are commonly used to replace one or more teeth. They eliminate the requirement to prepare the natural teeth adjacent edentulous areas, serve as abutments for fixed bridges, reduce the need for removable partial dentures, and add comfort and provide retention for complete dentures (American Academy of Periodontology 2008). As dental implants have become more ubiquitous in dentistry, a greater focus has been placed on the diagnosis and treatment of implant complications, namely peri-implantitis.

Peri-implantitis is defined as an inflammatory process of the tissues surrounding an osseointegrated implant in function, resulting in loss of supporting bone (Albrektsson et al. 1994). The prevalence of peri-implantitis is difficult to gauge. Studies vary with regard to the parameters used to define disease, subject selection and the length of observation (Mombelli and Lang 1998). The prevalence of peri-implantitis has been reported to range between 28-56% of subjects and 12-43% of implant sites.

There is an overwhelming body of evidence that supports a bacterial etiology for peri-implantitis. Several studies have demonstrated distinct qualitative and quantitative differences in the microflora associated with successful and failing implants. Successful implants tend to be colonized sparsely by gram-positive cocci, whereas failing implants harbor large amounts of gram-negative anaerobic bacteria (Rams and Link 1983, Mombelli et al. 1987, Mombelli et al. 1998, Sanz et al. 1990, Salcetti et al. 1997).

Early colonizers are Gram-positive bacteria. Some secondary colonizers are Gram-negative such as *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Capnocytophaga* species. The "tertiary colonizers" include *Porphyromonas gingivalis*, *Campylobacter rectus*, *Eikenella corrodens*, *Actinobacillus actinomycetemcomitans*, and the oral *Treponemes*. The source for bacteria associated with peri-implantitis is the oral biofilm, and individuals with poor oral hygiene and increased bacterial plaque are more susceptible to bone loss around implants (Lindquist et al. 1988). Further evidence of a link between plaque and peri-implantitis has been shown in animal studies demonstrating the experimental induction of bone loss around implants with plaque-retentive ligatures (Lang et al. 1993).

Surface debridement constitutes the basic element for treating both periodontitis and peri-implantitis (Lindhe and Meyle 2008). The ultimate goal in the treatment of peri-implantitis is re-osseointegration of the exposed implant surface utilizing a variety of methods including conservative, resective, and regenerative techniques used in conjunction with various methods of surface decontamination (Renvert et al. 2009). In recent years, the use of machined surface implants has diminished in favor of implants with a rougher surface texture (Alhag et al. 2008). The justification for the shift from smooth implant surfaces to more complex surface topography as seen in the NanoTite® design involves the desire for more rapid osseointegration and earlier loading of the implant (Buser et al. 1999). With these different implant surfaces comes variability in plaque retentive qualities and subsequent variation in the efficacy of commonly used disinfection modalities.

A majority of existing research focuses on the disinfection of previous generations of implants, hydroxyapatite-coated titanium and smooth, machined surface implants, which are used much less frequently today. In a 1992 study, Zablotsky et al. compared disinfection of hydroxyapatite-coated and grit-blasted implants. They reported that the different surfaces were not equally decontaminated by various chemotherapeutic agents or mechanical methods. Burnishing with citric acid for 1 minute and air-powder abrasion therapy proved to be superior for surface detoxification of hydroxyapatite-coated and grit-blasted surfaces, respectively. Zablotsky also showed that fibroblastic attachment to previously contaminated hydroxyapatite-coated implants was enhanced by citric acid application as compared to numerous other chemical agents and sonic scaling alone. Although air-powder abrasion has gained popularity in the treatment of peri-implantitis associated with roughened surface implants, its safety has been called into question due to reports of complications (Girdler 1994).

In 2010, Gosau et al. compared the ability of antimicrobial agents to both kill and reduce attachment of bacteria from human oral biofilms in an *in vitro* model utilizing a custom stent with embedded, machined titanium disks. Disinfection agents included 1% sodium hypochlorite, 3% hydrogen peroxide, 0.2% chlorhexidine gluconate, 0.3% triclosan, Listerine®, 40% citric acid, and a saline control. All agents significantly reduced the total number of attached bacteria after 1 minute of immersion.

The goal of peri-implantitis treatment is re-osseointegration of the affected implant, and previous studies have assessed the ability of various agents to aid in achieving such a goal. In a 2008 canine study, Alhag et al. demonstrated histologic evidence of re-osseointegration of roughened implants utilizing a 30 second citric acid application in the treatment of experimentally-induced peri-implantitis. In a critical review of the literature, Meffert (1996) concluded that the implant surface must be detoxified before regeneration of the supporting tissues is attempted, and this is best accomplished by the application of 40% citric acid, pH 1 for approximately 30 seconds.

Methods to treat inflammation and bone loss around implants mirror those used for disease around natural teeth (Meffert 1996). Several authors have employed traditional controlled-delivery devices to treat peri-implantitis. In 2001, Mombelli et al. showed overall improvements sustained for 1 year in clinical, radiological, and microbiological parameters in the treatment of 25 human patients with peri-implantitis utilizing tetracycline fibers. While there are no controlled studies that prove a benefit of tetracycline application in regeneration of lost periodontal tissues around natural teeth, an abundance

of empirical data exists and may serve as the motivation to evaluate the success of tetracycline in the treatment of peri-implantitis.

While the literature on peri-implantitis is filled with studies comparing various chemotherapeutic agents, the majority of the *in vivo* studies lack a standardized control group. Successful treatment of peri-implantitis has been shown by mechanical means alone in a 2009 study by Maximo et al. Peri-implantitis treatment via open flap debridement with Teflon curettes and air abrasion resulted in improved clinical parameters and decreased periodontal pathogens after 3 months (Maximo et al. 2009). The data from this study can be used to justify mechanical therapy alone as a standardized control when determining the possible added benefits of commonly used chemotherapeutic agents in the treatment of peri-implantitis. In 2003, Schou et al. compared air-powder abrasion alone to various combination therapies, including citric acid and chlorhexidine, for the treatment of experimentally induced peri-implantitis in monkeys. Histologic analysis of bone-to-implant contact (BIC) revealed similar values for all treatment modalities casting doubt on the necessity of using adjunctive chemotherapeutics to successfully treat peri-implant disease (Renvert et al. 2009). Similarly, Parlar et al. (2009) showed that utilization of an air-polishing device with sterile saline for 3 minutes resulted in considerable bone fill and re-osseointegration in mongrel dogs with experimental peri-implantitis. Froum (2011) published a case series involving treatment of 51 implants diagnosed with peri-implantitis. He proposed a stepwise approach to implant decontamination involving air abrasion for 2 minutes, tetracycline (50mg/mL) application for 30 seconds, chlorhexidine gluconate (0.12%) application for 30 seconds and copious sterile saline irrigation.

MATERIALS AND METHODS

This pilot study will use a modified methodology for sample preparation described by Kreisler et al. (2002). A total of 18 implants, 9 OSSEOTITE® and 9 Nanotite®, will be utilized. Customized models representing a circumferential bony defect typical of peri-implantitis lesions will be fabricated at the 3D Medical Modeling unit at WRNMMC using specific dimensions (Diagram 1.). The models (Figure 3.) will be fabricated with resin utilizing the Objet 500 Connex 3D printer. Each implant will be individually seated and inverted with the implant apex protruding from the resin model. The simulated defect, with a volume of 200uL, will serve as a well for contamination with a known early colonizer of oral biofilms.



Figure 3. Fabricated resin model of a circumferential bony defect.

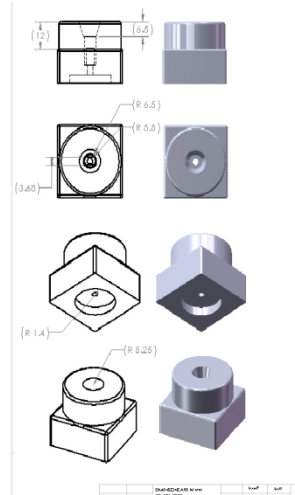


Figure 4. Implant model design

Streptococcus sanguinis will be sub-cultured in Brain Heart Infusion broth (BHI; 37°C, 5% CO₂, 95% humidity). After 24 hours of incubation, 0.9 mL of the BHI culture will be transferred to 44.1 mL of fresh, pre-warmed Biofilm Media (BM) and incubated (37°C, 5% CO₂, 95% humidity) to formulate the implant contamination media. The bacteria will be grown to an optical density (Genesys 10-5) of 0.5 at 600nm. This will yield a concentration of 1.5×10^8 Colony Forming Units per milliliter. Bacterial growth will be arrested by placing the sample preparation media on ice.

Eighteen Biomet 3i™ implants [9 tapered, internal OSSEOTITE® and 9 NanoTite® (5 x 13mm)] will be used. Individual implants will be identified by numbering from 1 to 18. Each category of implant will be divided into treatment (n=5), positive control (n=2) and negative control (n=2) groups.

All implants will be individually mounted and numbered in 18 sterilized resin models. The models will serve as testing units and will be assembled using sterile technique by first placing a sterile impression coping into the model. The implant will then be seated into the coping, and a coping screw will be hand tightened to secure the implant/coping in the model, forming a hermetic seal at the defect base. A leak test using methylene blue dye for 24 hrs was performed and confirmed an adequate seal.

CONTAMINATION PHASE

For treatment (n=9) and positive control (n=2) groups of both implant categories, 200uL of suspended contamination media will be pipetted into the simulated bony defect. Negative controls (n=2) will be incubated with sterile Biofilm Media (200uL). Approximately 5 implant threads will remain

exposed above the submerged portion. These exposed threads will be used to facilitate handling for decontamination procedures in an effort to minimize cross-contamination. Following contamination, all samples will be incubated at 37°C, 5% CO₂ and 95% humidity for 24 hrs. Following incubation, the broth will be removed by careful suctioning, and the implants will undergo immediate decontamination.

DECONTAMINATION PHASE

Treatment groups will undergo a 1 minute decontamination process as described by Fosie (2011). A sterile cotton pellet saturated with a tetracycline (50mg/mL) solution will be wiped circumferentially with standardized force for 1 minute (20 times/min) around all contaminated implant surfaces.

Positive controls will not undergo decontamination. Negative controls will undergo the same decontamination procedure as the treatment groups with the exception that only sterile saline will be used to saturate the sterile cotton pellet.

Following the decontamination phase, all implants will be removed from their resin model using sterile technique. The individual implants will be placed into a 15 mL centrifuge tube containing 2.0 mL of phosphate buffered saline (PBS). All tubes will be vortexed using a digital multi-tube (Fisher Scientific) vortex machine (1 min, 1000rpm) to dislodge any remaining bacteria. Following agitation, the samples will be placed in an ice bath to suspend growth.

Each sample will be diluted to 1:10 and 1:1000 with cold, sterile PBS and vortexed. A 0.05mL aliquot from each sample will be streaked on BHI agar in triplicate and incubated (37°C, 5% CO₂, 95% humidity) for 24 hours. Following incubation, each plate will be counted and the number of CFUs will be recorded for comparison.

RESULTS

A clinically relevant in vitro model of peri-implantitis has been created. No experimental results are available at this time, but SRB approval and funding has been realized. LCDR Scott Pasieta will be the new PI for this project which will be guided by the same research team.

DISCUSSION

While there are no results from which conclusions can be made, the ability to decontaminate OSSEOTITE and Nanotite surfaces with mechanical tetracycline applications may differ due to the differences in surface topography of the implants. The simulated intrabony defect model incorporated into this experimental design heightens the validity of this in vitro study. If the results of this study show that increased surface area and complexity of implant surfaces harbor more live bacteria after mechanical disinfection, perhaps such increased surface complexities of dental implants enhance the risk for peri-implant mucositis and peri-implantitis. This novel in vitro model may have a variety of applications in studying implant decontamination protocols among differing chemotherapeutic and mechanical methodologies. Implant systems can also be compared along with an increasingly complex mixture of bacteria.

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