THE EVALUATION OF TITANIUM SHEDDING FROM IMPLANTS WITH THREE DIFFERENT SURFACE TEXTURES

A Thesis

by

JAMES MICHAEL WOMACK

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Carlos Parra
Co-Chair of Committee,	Stephen Harrel
Committee Members,	Paras Patel
Head of Department,	Madhu Nair

May 2021

Major Subject: Oral Biology

ABSTRACT

The purpose of this investigation was to assess the effect of implant surface treatment on titanium particle shedding as a measure for peri-implantitis potential following piezoelectric instrumentation. Three groups of five implants with different surface treatments were assessed: machined surface, sandblasted acid etched (SLA), and anodized. A piezoelectric scaler with a titanium tip was placed against the coronal one third of the body of the implant using 25 grams of force and cycled for 30 strokes. Water containing any titanium particulate was collected and stored in a centrifuge tube. One implant from each group was used as an active control. The collected water was centrifuged and evaporated. Particles were then re-suspended in a known volume of water (0.1 ml). Calculation of titanium particles was determined by pipetting a fixed volume (10 µl) of the standardized solution into a hemocytometer. The presence of titanium was confirmed via elemental analysis.

All implants used within the experiment released titanium particles. The mean particle count for the anodized group was (11,333), machined group (8,333), and the SLA group (7,633). Tukey's test revealed implants with the anodized surface released a statistically significant larger number of particles as compared to the SLA surface with a p-value = 0.0245. No statistically significant difference in particle count was noted between the SLA surface or the machined surface. There was no statistically significant difference between the machined and anodized surface, however, there was a trend toward a larger number of particles being produced in the anodized group. All implants in the active control group were also found to release modest amounts of particles anodized (933), machined (533), and SLA (866). The light microscope and scanning electron microscope revealed variations in the size of titanium particulate with particles as small as two microns being identified.

Elemental analysis confirmed the presence of metallic particulate to be titanium particles.

The results from this study suggest the anodized surface, when instrumented with a titanium piezoelectric tip, release a larger quantity of particles as compared to the SLA implant surface.

DEDICATION

This thesis is dedicated to my mother for her steadfast love and caring throughout my life. To my wife for her consistent support throughout the course of our journey together. To my brother, relatives, friends, faculty, and mentors who have provided their advice and encouragement during the pursuit of my master's degree.

ACKNOWLEDGEMENTS

I cannot express enough thanks and gratitude to my master's thesis committee members, Dr. Harrel, Dr. Carlos Parra, and Dr. Paras Patel for their efforts in supporting me throughout the master’s process. I would also like to thank Dr. Thomas Diekwisch, Dr. Xianghong Luan, and Dr. Mirali Pandya for their continual guidance throughout the laboratory portion of the experiment. Thank you to Dr. Jorge Gonzales for donating machined implants to this study. I would like to extend my gratitude to Dr. Panagiotis Afouxinedes, Dr. Sarah Atwi, and Dr. Elias Kontogiorgos for their contribution in the statistical analysis portion of my findings. Finally, thank you to Dr. Pooria Fallah along with the staff and residents of the Texas A&M Graduate Periodontics Department for their encouragement and support throughout the process.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supported by a dissertation committee consisting of Dr. Carlos Parra (chair) and Dr. Stephen Harrel (co-chair) of the Department of Periodontics, and Dr. Paras Patel of the Department of Diagnostic Sciences.

Laboratory processes in the form of titanium particle processing and identification via light microscopy and SEM was provided by Dr. Thomas Diekwisch and Dr. Mirali Pandya. Titanium particle collection was conducted with assistance from Dr. Stephen Harrel and Dr. Panagiotis Afouxinedes. Statistical analysis was performed with the assistance of Dr. Panagiotis Afouxinedes, Dr. Sarah Atwi, and Dr. Elias Kontogiorgos. The student completed all other work conducted for the thesis independently.

Funding Sources

Brånemark machined implants were donated by Dr. Jorge Gonzales. This investigation was funded by a thesis research grant provided by the Texas A&M University College of Dentistry. This project was also supported by the Graduate Periodontics Department.

NOMENCLATURE

ANOVA	Analysis of variance
BIC	Bone to implant contact
HCl/H2SO4	Hydrogen Chloride/Sulfuric Acid
IL-1	Interleukin-1
IL-1B	Interleukin-1B
IL-6	Interleukin-6
IL-8	Interleukin-8
ITI	International Team for Implantology
MMP-2	Matrix metalloproteinase-2
MMP-9	Matrix metalloproteinase-9
mRNA	Messenger Ribonucleic Acid
ppm	Parts per million
PSI	Pounds per square inch
RANKL	Receptor activator of nuclear factor-κB ligand
RANKL/OPG	Receptor activator of nuclear factor-κB ligand/Osteoprotegerin
SEM	Scanning Electron Microscopy
SLA	Sandblasted large grit acid etched
Ti	Titanium
Ti-6Al-4V	Titanium, aluminum, and vanadium
TiZr	Titanium zirconium
TGF-α	Transforming growth factor-alpha

TGF-β	Transforming growth factor-beta
TNF-α	Tumor necrosis factor-alpha
VEGF-A	Vascular endothelial growth factor A

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Within the past 50 years, the dental implant has grown from a relatively experimental treatment modality to a restorative option that is both successful and predictable (1). The increase in demand by patients for dental implants has spurred a rise in the number of dentists receiving training in implant placement (2). A trend toward increasing implant procedures is reflected in the market research which shows a growing number in dental implant sales (3). The increased popularity of dental implants is attributed to many advantages including the ability to offer a restorative solution which can closely mimic what would be seen in the natural dentition. Additionally, dental implants have been shown to significantly increase masticatory function in the fully and partially edentulous patient (4), (5), (6). In the United States, it is projected that dental implant prevalence could reach as high as 23% by the year 2026 (3).

The Evolution of Implants

The replacement of teeth with an endosseous implant was first seen in 600 A.D. within the Mayan culture where pieces of seashells were used to replace missing mandibular incisors. As humans evolved, so did the advances in dental implantology. In the 1800's, clinicians began experimenting with various materials that could be implanted in the jaw to replace a missing tooth. These materials included gold, silver, porcelain, and iridium. However, it was not until the twentieth century that the first successful dental implants were being placed (7). During the 1940's, Doctors Alvin and Moses Strock used Vitallium orthopedic screws to restore missing dentition (8). As the knowledge and understanding of dental implantology evolved, so did the development of various dental implant designs and materials. From the 1940's through the 1970's, dental implants took on many forms, from a blade shape, subperiosteal frameworks, transmandibular devices and variations on a helical spiral form (9). Implants that are commonly seen today are titanium endosseous root form implants (10). Much of the design of the dental implants we know today can be attributed to the work of two pioneers in dental implantology, Dr. Per-Ingvar Brånemark and Dr. André Schroeder. After early animal studies looking at the interaction between titanium and bone, Dr. Brånemark developed a threaded root-form implant which was made of titanium (11). In the 1960's, he began placing these implants in humans to provide a platform for the restoration of the completely edentulous arch. In 1977, he published convincing and well documented ten-year data of his titanium dental implants used in the oral rehabilitation of edentulous patients (12). Dr. Schroeder, along with help of the International Team for Implantology (ITI), also furthered the research and development of many early root form endosseous implants (13). The initial Straumann implant differed from Brånemark's machined implant in that it utilized a roughened titanium plasma sprayed coating, was available with or without screw threads, and had a transmucosal collar (14), (15). The initial progress in dental implant design achieved by Schroeder and the ITI ultimately led to the development of the Straumann tissue level implant that is currently available. The foresight of these two innovators in the field of dental implantology helped shape dental implants as we know them today.

Osseointegration

The detection of osseointegration between titanium and bone was first described in the animal studies conducted by Bothe, Beaton, and Davenport in 1940, who observed the tendency of titanium to fuse with bone (16). This same phenomenon was later described by Leventhal in 1951, who found titanium screws implanted in the femur of rats were difficult to remove. Dr. Leventhal noted that at 16 weeks after implantation, the bond formed between titanium and bone of one specimen was so strong that the femur fractured on screw removal (17). The same adhesion between titanium and bone was also noted by Per-Ingvar Brånemark in the 1950's (18). During his initial animal studies on circulation and healing, it was found that the titanium chambers which had been placed in the femurs of rabbits became impossible to remove without subsequently damaging the neighboring bone (18), (19). Dr. Brånemark further studied the phenomenon of bone growth to titanium more in depth by placing titanium screws into the femurs of rabbits and had hollow glass chambers attached to the implant (11). The glass chamber allowed him to physically see how the blood vessels, along with bone, interacted with the titanium surface. His findings contributed to a better understanding of what we now know as osseointegration. It wasn't until 1977 that the term osseointegration was introduced into the dental vernacular (12). The term osseointegration was later defined as "a direct structural and functional connection between ordered living bone and the surface of a load-carrying implant" (20).

Titanium/Titanium Alloys

Titanium, along with its alloy derivatives, have both biologic and mechanical properties which make it a desirable material to be used in the field of implantology (21). Dental implants typically consist of either commercially pure titanium or a titanium alloy. Commercially pure titanium is available in four grades. These four grades of titanium are distinguished by the differences in quantity of their trace elements of oxygen, carbon, and iron (22), (23). This difference in trace elements translates into varying degrees of tensile strength between the four classes of commercially pure titanium, with grade 4 having a tensile strength more than two times that of grade 1 pure titanium (24). In applications subject to high stress, the strength of a commercially pure titanium implant may be considered inadequate (25). In an effort to increase implant strength, titanium alloys have begun to be used in dental applications. Titanium alloys comprised of titanium, aluminum, and vanadium (Ti-6Al-4V) or alloys containing titanium and zirconium (TiZr) have gained popularity in dental implantology (25), (26). The overarching goal of the use of titanium alloys in dental implantology is to maintain biocompatibility while also maximizing the strength of the implant. The Nobel Biocare company currently uses a commercially pure grade 4 titanium for implants and Ti-6Al-4V for their abutments and cover screws (27). The Straumann company offers implants in a commercially pure grade 4 titanium, or in a TiZr alloy which allows for increased strength (27).

Dental Implant Surface Modification

Sa value is a frequently used parameter to quantify dental surface roughness into a numerical value (27). The Sa value is an arithmetic mean of the roughness area from a mean plane that describes height deviation and amplitude of the implant surface topography (27), (28). Implants have been classified by their Sa value as either being smooth Sa < 0.5 µm, slightly rough Sa 0.5-1 µm, moderately rough Sa 1-2 µm and very rough Sa > 2 µm (29). Early Brånemark implants were fabricated from commercially pure titanium and had machined surfaces. Although these machined implants visually appear polished, the lathe turning fabrication process produces a titanium surface that can be described as slightly roughened (30). Even though a moderately roughened surface is considered to induce a more favorable bone response, the early slightly rough machined surface was still found to have favorable results. In fact, many studies which have compared implant success generally find no significant difference between the two surfaces (30). One major drawback to the use of machined surface implants was a longer wait time of three to six months prior to loading (12) This timeframe is much different when compared to moderately roughened surfaces that can follow an early loading protocol of as little as six weeks (31). Dental implant surface treatments increase surface roughness and have been shown to be beneficial at increasing bone to implant contact (BIC) as well as improving time for osseointegration (32), (33), (34). Cell differentiation, cell alignment, and osteoblast proliferation have all been shown to improve with a moderately rough surface (35), (36). Unfortunately, the same cannot be said for implants with a very rough surface. Implants with a very rough surface have fallen out of favor due to increased incidence of peri-implant mucositis and peri-implantitis (37).

Surface modification treatments can be divided into two broad categories based on the method used to create a roughened surface. These two categories of surface treatments can be described as either additive or subtractive (38). Examples of additive methods of creating a roughened dental implant surface include plasma sprayed hydroxyapatite coating or surface modification with titanium plasma spray. These early methods of implant surface modification were found to produce a surface that was classified as very rough (39). Implants with very rough surfaces were found to have significant increases in implant failure and marginal bone resorption shortly after insertion (40), (41).

Subtractive or reorganizational surface treatments currently used on dental implants are commonly created via grit blasting, acid etching, anodization, and laser etching (22), (38), (42).

These surface modifications produce a moderately rough surface which have demonstrated favorable well documented success and survival rates (1). One company that utilizes an anodized implant surface is Nobel Biocare (27). An anodized surface can be created by submerging the titanium implant into an electrolyte bath. Once in the bath, the implant will act as an anode; an electric current is then applied to the solution at varying intensities (43). At lower voltage, the deposition of an oxide layer occurs along the implant surface. At peak electrical intensity, a process of spark anodization occurs on the outer portion of the implant, resulting in a porous moderately roughened implant surface (43). Another popular method to produce a moderately rough surface occurs through either sand blasting, acid etching, or a combination of sandblasting and acid etching. One company that utilizes a sandblasted and acid etched surface on their dental implants is Straumann (27). This type of surface modification is created by first grit blasting the titanium implant with large grit (0.25-0.50 mm) particles of alumina, followed by etching in a mixture of HCl/H_2SO_4 (44) and is abbreviated as SLA surface. Both the anodized surface and the sandblasted acid etched surfaces have been shown to produce favorable long-term results with improved benefits in BIC, higher removal torque, and reduced healing times when compared to early machined implants (45), (46), (42).

Peri-Mucositis/Peri-Implantitis

The high rate of success of titanium dental implants has revolutionized the field of restorative dentistry (1). Early on, Albrektsson *et al.* developed a criterion of five points which should be met to consider an implant successful. These five criteria are as follows: 1.) the implant is non-mobile 2.) absence of peri-implant radiolucency 3.) vertical bone loss less than 0.2 mm per year after the first year of placement 4.) absence of pain, infection, neuropathies, paresthesia, or violation of the mandibular canal 5.) a minimum 85% success rate at the end of a 5-year observation period and 80% at the end of a 10-year period (47). Numerous studies have shown 10-year success and survival rates of dental implants to be in the 95th percentile (48), (49), (50). However, despite its positive long term track record, dental implants may still become diseased. Implants can be categorized as having two diseased states: either peri-implant mucositis and/or peri-implantitis. A meta-analysis by Derks estimated the prevalence of peri-implant mucositis and peri-implantitis to be 42.9% and 21.7% (51). The characteristics of these two disease states have been described and classified by numerous authors throughout the years including Lindhe & Meyle 2008, Lang & Berglundh 2011, and Sanz & Chapple 2012 (52), (53), (54).

Peri-implant mucositis and peri-implantitis are distinguished by the extent of their inflammation and subsequent destruction of the supporting tissues. More recently, a classification of dental implant diseases and conditions was defined at the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Peri-implant health is characterized by the absence of erythema, bleeding on probing, swelling, and suppuration (55), (56). Peri-implant mucositis exhibits bleeding on probing along with erythema, swelling, and/or suppuration (56), (57). In peri-implantitis, tissues surrounding the dental implants are again characterized by inflammation in the peri-implant mucosa, but also display the additional progressive loss of supporting bone. Instances of peri-implantitis may show clinical signs of inflammation, bleeding on probing, and/or suppuration, increased probing depths, and/or recession of the mucosal margin, in addition to radiographic bone loss (56), (58).

The primary etiology of these two disease states is generally considered to be microbial in nature (59). Endotoxins produced by bacteria promote a proinflammatory state which subsequently leads to the activation of osteoclasts, resulting in peri-implant bone loss. However, within the dental literature, there is evidence that links peri-implantitis with risk factors such as excess cement and implant placement that does not allow for adequate oral hygiene (56), (59). Additionally, within the 2017 classification, peri-implantitis risk indicators were identified. Risk indicators are topics which have been associated with peri-implant disease, however, they are based off a lower level of evidence i.e., cross sectional and case control data (60). These risk indicators outlined by Berglundh *et al.* include keratinized mucosa, occlusal overload, bone compression necrosis, overheating during implant placement, micromotion, biocorrosion, and the presence of titanium particles in the surrounding peri-implant tissue (56).

Titanium Particles

The concept of metal particles being associated with prothesis failure is not new and has previously been identified in medical literature as early as 1970 at sites using fixation screws for the repair of fractures (61). Within the surgical orthopedic literature, there is a growing prevalence of prothesis failure that can be attributed to the accumulation of metal particles adjacent to prosthetic joints (62). The inflammatory response which occurs as the result of the accumulation of titanium particles is described as a condition called metallosis. In its simplest form, metallosis can be defined as the buildup of metal debris within the tissues of the body (63). A build-up of metal particles around a prosthetic joint has been shown to have the potential to result in inflammation and osteolysis, which may ultimately result in failure of a prosthesis (64).

Within the dental literature, the idea that peri-implantitis and/or peri-implant mucositis may be related to metallosis is beginning to gain more attention (65). As a result of its high success rate, the dental implant is considered by many to be the go-to option for tooth replacement. The original Brånemark titanium root form dental implants consisted of a threaded titanium screw with a machined surface. Advances in the understanding of osseointegration and surgical implantology lead to the development of moderately roughened surface root form implants that are routinely used today. Roughened surface implants provide more surface area, thus improving overall BIC. However, concerns have been raised about the detachment of microscopic titanium particles from the roughened surface of dental implants (66). It has been shown that titanium particles may be released at implant placement, during routine cleaning, and even while in function (66), (67), (68). It has been speculated that detached titanium particles may play a role in the development of peri-mucositis and peri-implantitis via an inflammatory reaction in the surrounding peri-implant tissues (69).

Titanium oxide is commonly found in day-to-day products including toothpastes, sunscreen, and even food (70). Although it is possible for titanium particles to be ingested, in patients with dental implants, the titanium particles may be the result of shedding of the titanium from the implant surface. Weingart *et al.* found titanium particles along the peri-implant tissues and within regional lymph nodes of beagle dogs that had dental implants placed in osteotomies which were not tapped (71). He *et al.* assessed the levels of metallic particulate in the mandibular bone of subjects who had previously received dental implants. In this study, levels of titanium were determined to be significantly higher in patients with dental implants as compared to patients without dental implants (72). Additionally, an animal study conducted by Meyer *et al.* revealed the process of implant insertion led to the deposition of titanium particles within the peri-implant bone (73).

With regard to the effect of metallosis on bone, various studies have discussed the effect that titanium particles may have on osteoblasts. The literature suggests the quantity of titanium particulate may play a factor in the disruption of cellular biologic processes. An *in vitro* study conducted by Pioletti *et al.* described the cytotoxic effect of titanium particle concentration on osteoblasts. Results of the experiment revealed a direct correlation between osteoblast viability and titanium particle concentration. In this study, titanium particles had a direct effect on osteoblasts by inducing apoptosis. Additionally, an indirect effect was noted in that osteoblasts which had phagocytized titanium particles released cytotoxic biproducts (74). In addition to these findings, Wachi *et al.* showed in a rat model that titanium ions in a concentration of 9 ppm significantly increased mRNA expression, chemokine ligand 2, and the ratio of RANKL/OPG (75).

When discussing titanium particles and their effects on osteoblasts, the size of titanium particulate should also be considered. The literature suggests titanium particle size is influential in the osteoblast viability and in initiating a cellular response. A 2005 article by Choi *et al.* discussed the effects that titanium particle size has on osteoblast viability. Results of this study indicated all sizes of titanium particles that were tested, when phagocytized, resulted in decreased osteoblast adhesion and proliferation. Interestingly, titanium particles > 1.5μm were shown to increase the expression of RANKL (76). A study by Kumazawa *et al.* investigated the cytotoxicity of particulate titanium (1-3 μm compared to 10 μm size) in human neutrophils. Results revealed increased superoxide anions and TNF-α levels when neutrophils were exposed to a solution of titanium particles with a smaller size of 1-3 μm. The increase in TNF-α resulted in neutrophil activation and subsequent inflammation (77). This adds support to the notion that the size of titanium particles is a key factor in initiating a cellular response.

Histologic analysis of the soft tissue surrounding failing implants has shown fibroblasts may react adversely to titanium particles. A study by Wei *et al.* showed an increased expression of

RANKL from synovial derived fibroblasts which had been stimulated with titanium particles 1-3 μm in size (78). Irshad *et al.* assessed the inflammatory response of peri-implant granulation tissue fibroblasts to titanium by itself and combined with *P. gingivalis*. It was determined that exposure to titanium particles alone resulted in increased expression of TNF-α and an increase in protein production of TNF- α, IL-1B, IL-6, and IL-8. Results also showed that titanium particles in the presence of *P. gingivalis* resulted in a greater increase in gene expression of TNF-α, protein production of TNF-α, and monocyte chemoattractant protein-1 as compared to *P. gingivalis* alone. It was ultimately concluded that titanium particles, along with the presence of *P. gingivalis* may play a role in the progression of peri-implantitis by enhancing inflammation in peri-implant soft tissues (79).

CHAPTER II

STUDY DESIGN

Purpose

To calculate titanium particle shedding from three dental implants with different surface modifications (machined, SLA, anodized) that have undergone instrumentation with a piezoelectric scaler using a titanium tip.

Null Hypothesis

Implants that have undergone different surface modifications (i.e., machined, SLA, anodized) will have no difference in the quantity of titanium particles shed when instrumented with a piezoelectric scaler with a titanium tip.

Clinical Reasoning

There are many studies which have drawn an association between titanium particulate and the inflammatory response surrounding the peri-implant soft tissue of failing implants. Wilson *et al.* looked at histologic sections of tissue surrounding failing implants, which revealed the presence of titanium fragments embedded within the soft tissues (69). Light microscopy revealed a mix of subacute and chronic inflammation predominated by plasma cells, which were found to be in close approximation to the titanium fragments embedded within the surrounding soft tissue of failing implants. Additionally, Fretwurst *et al.* noted increased lymphocytes and macrophages in peri-implant tissues that contained metallic particles, however, no correlation could be drawn between titanium particle quantity and number of macrophages (80). Findings from Olmedo *et al.* noted higher concentrations of titanium particles within exfoliated cells next to implants with peri-implantitis as compared to samples harvested from healthy sites (81). A more recent study by Safioti *et al.* revealed larger quantities of titanium particles within submucosal biofilm on implants with peri-implantitis as compared to healthy controls (82).

It is suggested that these titanium particles and their associated inflammatory response may play an etiologic role in the development of peri-implantitis. An *in vitro* study by Harrel *et al.* evaluated titanium particle release, which resulted from various modes of ultrasonic instrumentation on dental implants with an SLA surface (66). Findings from this study demonstrated titanium particles were released as the result of ultrasonic instrumentation as well as via water spray control. However, this study does not account for particle release from dental implants with different surface textures and only investigated instrumentation of implants with an SLA surface. The manner in which a roughened titanium surface is produced varies from manufacturer to manufacturer, with much of this processing information being proprietary. Currently, implant companies employ vastly different methods for creating their roughened surface implants (27). Nobel Biocare implants undergo a surface treatment in which the roughened surface is created by way of an anodization process (TiUnite^{TM} surface). While the Straumann implant surface is created via sandblasting with aluminum oxide particulate followed by acid etching (SLA surface) (44). To date, there is no information regarding the quantity of titanium particle release that occurs from implants with machined, anodized, and SLA surface modifications as the result of piezoelectric instrumentation. It is reasonable to assume surface texture may play a part in titanium fragment quantity and size. Therefore, the purpose of this experiment was to assess the effect of implant surface treatment on titanium particle shedding as a measure for peri-implantitis potential following instrumentation using a piezoelectric scaler with a titanium tip.

Materials and Methods

A total of 15 implants were used in the study. Three different groups consisting of five implants with differing surface treatments were assessed: machined surface (Nobel Biocare, Mark III^{TM}), SLA (Straumann, Standard Plus^{TM}), and anodized (Nobel Biocare, NobelSpeedy Replace^{TM}). A reciprocating holding device (Figure 1) was used to move the implants against the piezoelectric scaler (Acteon, NEWTRON P5 XS^{TM}) with a titanium tip specifically designed for implant care (Acteon IP2R).

Figure 1. Reciprocating holding device.

The piezoelectric scaler reservoir was filled with deionized water and irrigation lines were purged prior to conducting the experiment; three samples of the purged deionized water were collected and assessed for contamination of the water line and water reservoir. During the experiment, the titanium piezoelectric tip was placed against the coronal one third of the body of the implant, Figure 2. The titanium tip contacted each implant with a pressure of 25 grams of force. Force measurements were calculated via a digital scale placed beneath the implant. Each implant was cycled for 30 strokes, Figures 2,3,4. The water pressure (drip by drip) and power settings (power setting 5) were adjusted to the manufacturers recommended setting for all implants that were tested. Water containing any titanium particulate was collected into a glass funnel (Pyrex short stem 60-degree angled funnel) and stored in sterile 50 ml polystyrene conical centrifuge tubes (SPL Life Sciences).

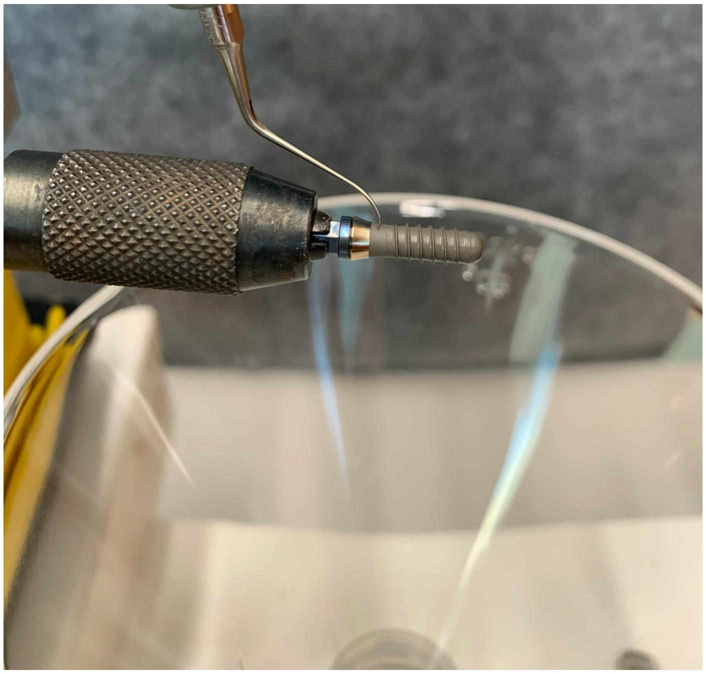

Figure 2. Piezoelectric tip in contact with implant.

Figure 3. Implant post instrumentation

Figure 4. Anodized, SLA, and machined surface implant post instrumentation.

One implant from each group was used as a control. Implants in the control group were secured onto the holding device as previously described. However, during this portion of the experiment, the tip of the piezoelectric scaler was placed 5 mm from the body of the implant. Water pressure from the titanium piezoelectric tip was utilized to rinse the implant (PSI of water pressure exerted from a piezoelectric scaler on manufacturer recommended power setting). The implant was cycled in the same manner as used in the experimental section, but no instrumentation was performed, i.e., water lavage only. Water was collected in a centrifuge tube using the same method described earlier. All implants were tested under duplicate conditions along the three different implant surfaces.

The collected water was centrifuged at 1800 rpm for 1 hour and then evaporated. The particles were then re-suspended in a known volume of water (0.1 ml). This provided a standardized volume of solution for all samples and allowed for the assessment of particle concentration within groups. Calculation of titanium particles was determined by pipetting a fixed volume (10 μl) of the standardized solution into a Bright-Line Hemocytometer (Hausser Scientific). The hemocytometer has a reservoir that holds a standardized volume of solution which allowed for the calculation of particles in a given volume. Particles were assessed on 10x magnification (Leica DM IL), Figure 5.

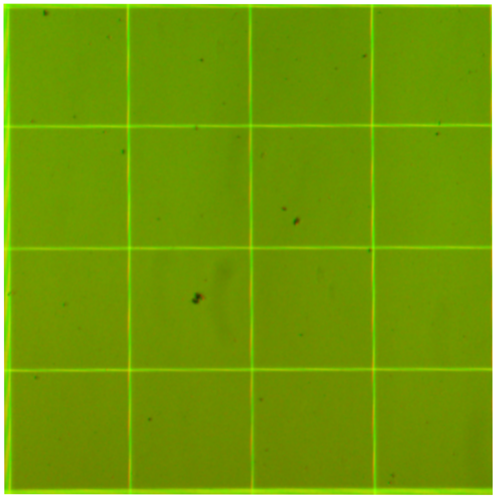

Figure 5. Particle counting on hemocytometer (10x).

Implant particles were assessed via light microscopy at 10x magnification (Leica DM LB), Figure 6. Counts were conducted three times per sample with the mean being used as the final particle count. Additionally, metal particulate size and composition were assessed via SEM and presence of titanium was confirmed via elemental analysis using point energy dispersive x-ray spectroscopy (JEOL JSM-6010LA), Figures 7, 8, 9. Additionally, the implant body was evaluated via light microscopy for alterations to the portion of the implant which was instrumented.

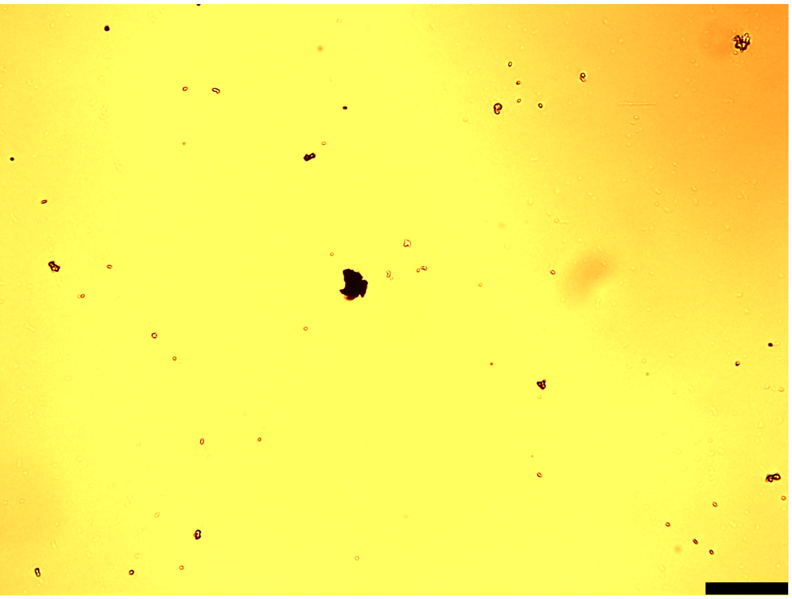

Figure 6. Light microscopy of metallic particles within the collected sample (10x).

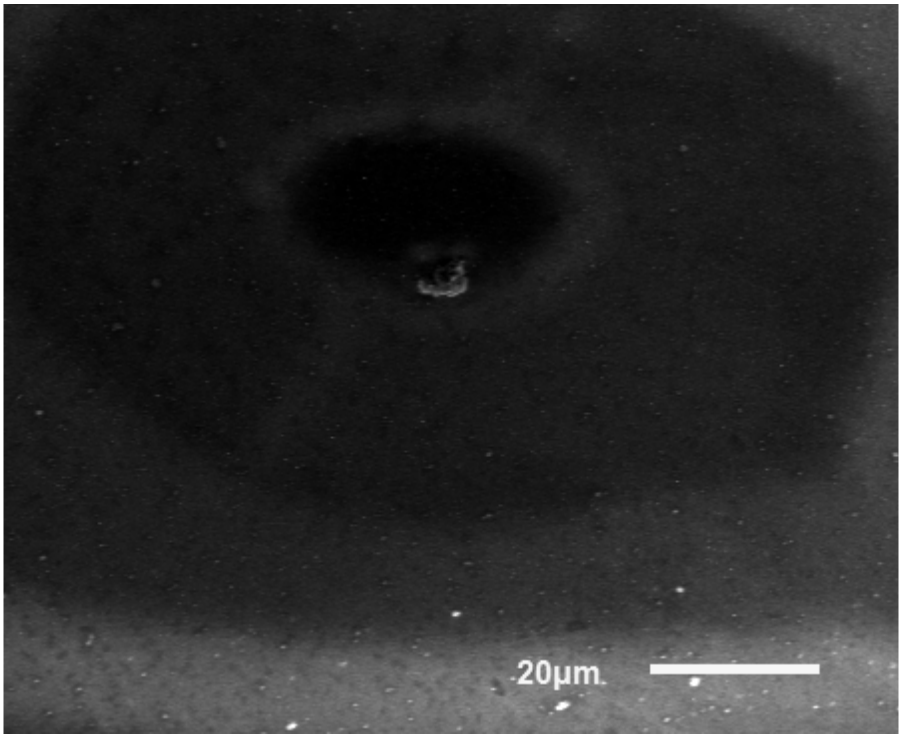

Figure 7. SEM of metallic particles within the collected sample.

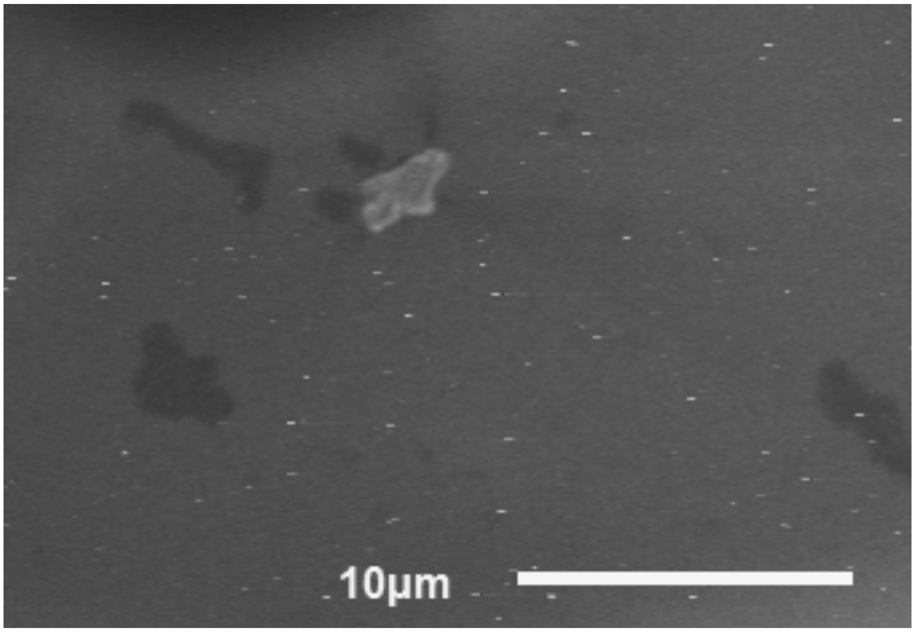

Figure 8. SEM images of metallic particle within the collected sample.

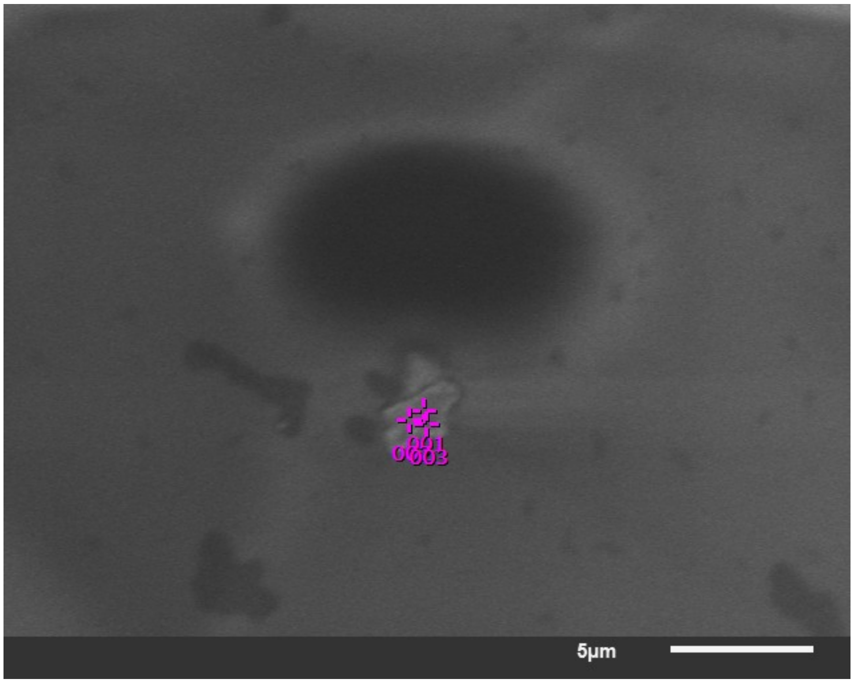

Figure 9. Particle selection for energy-dispersive X-ray spectroscopy.

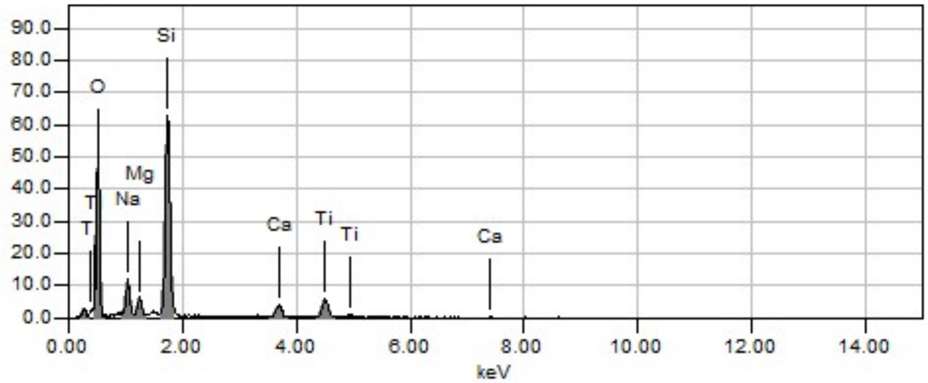

Figure 10. Elemental analysis identifying titanium within the sample.

CHAPTER III

RESULTS

Results from this study indicate all implants used within the experiment released titanium particles. The mean particle count for the anodized group was (11,333 ± 333.15), machined group (8,333 ± 1,347.51), and the SLA group (7,633 ± 2,243.48), Table 1, Figures 11, 12. Data analysis performed with an ANOVA test revealed $p < 0.05$, Table 2. The null hypothesis that there is no statistically significant difference between the groups was rejected. Post hoc Tukey's test revealed implants with the anodized surface released statistically significant larger amounts of particles as compared to the SLA surface, p-value = 0.0245, Table 3. No statistically significant difference in particle count was noted between the SLA surface or the machined surface. There was no statistically significant difference between machined and anodized surface, however, there was a trend toward a larger number of particles being produced in the anodized group, p-value = 0.0671, Table 3. Additionally, all implants in the active control group (i.e., water lavage only) were also found to release modest amounts of particles as compared to the experimental group anodized (933), machined (533), and SLA (866), Table 4. The light microscope and scanning electron microscope revealed variations in the size of titanium particulate that was shed from the implant surface with particles as small as 2 µm being identified. Elemental analysis via point energy dispersive x-ray spectroscopy confirmed the presence of metallic particulate to in fact be titanium particles within the samples, Figure 10.

Data Summary				
Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**
Anodized	4	11133	331.1485	165.5743
Machined	4	8333	1347.5135	673.7568
SLA	4	7633	2243.4762	1121.7381

Table 1. Data summary of group means values.

ANOVA Summary					
Source	**Degrees of Freedom DF**	**Sum of Squares SS**	**Mean Square MS**	**F-Stat**	**P-Value**
Between Groups	2	27440000	13720000	5.915	0.0229
Within Groups	9	20875912.2651	2319545.8072		
Total:	11	48315912.2651			

Table 2. ANOVA Summary.

	Group 1	Group 2	Diff	Lower	Upper	q-value	P-value
0	Anodized	Machined	2800.25	-204.034788	5804.534788	3.677253	0.067185
1	Anodized	SLA	3500.25	495.965212	6504.534788	4.596484	0.024409
2	Machined	SLA	700.00	-2304.284788	3704.284788	0.919231	0.784920

Table 3. Post hoc analysis.

Active Control		
Groups	**N**	**Mean**
Anodized	1	933
Machined	1	533
SLA	1	866

Table 4. Data summary of active control group mean values.

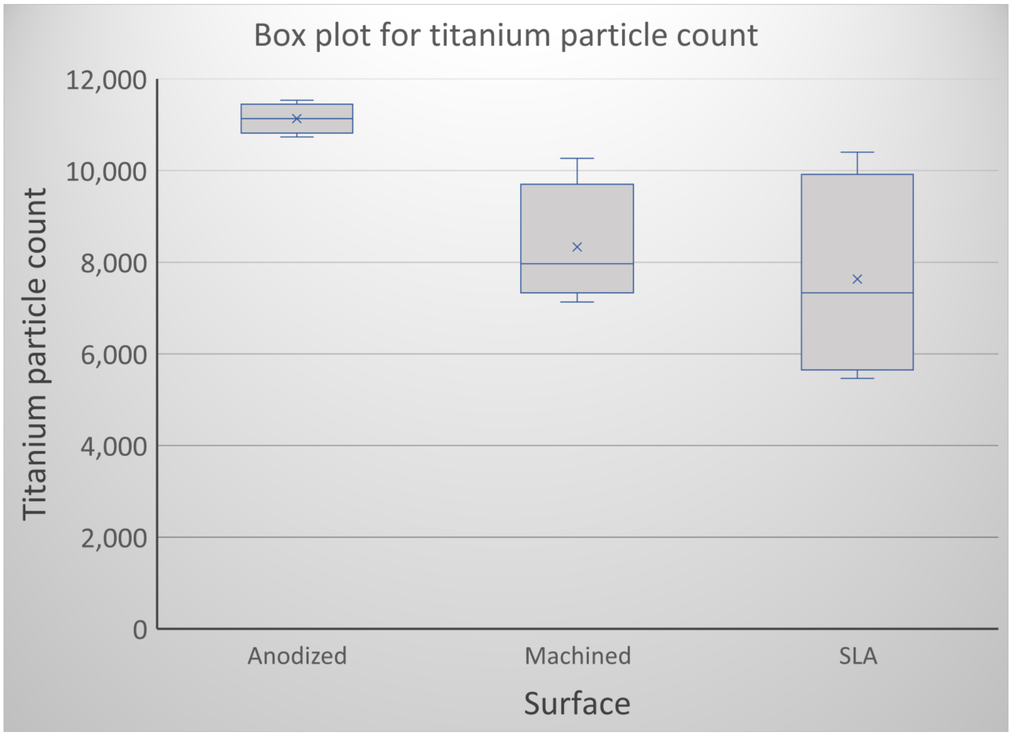

Figure 11. Box plot for titanium particle count.

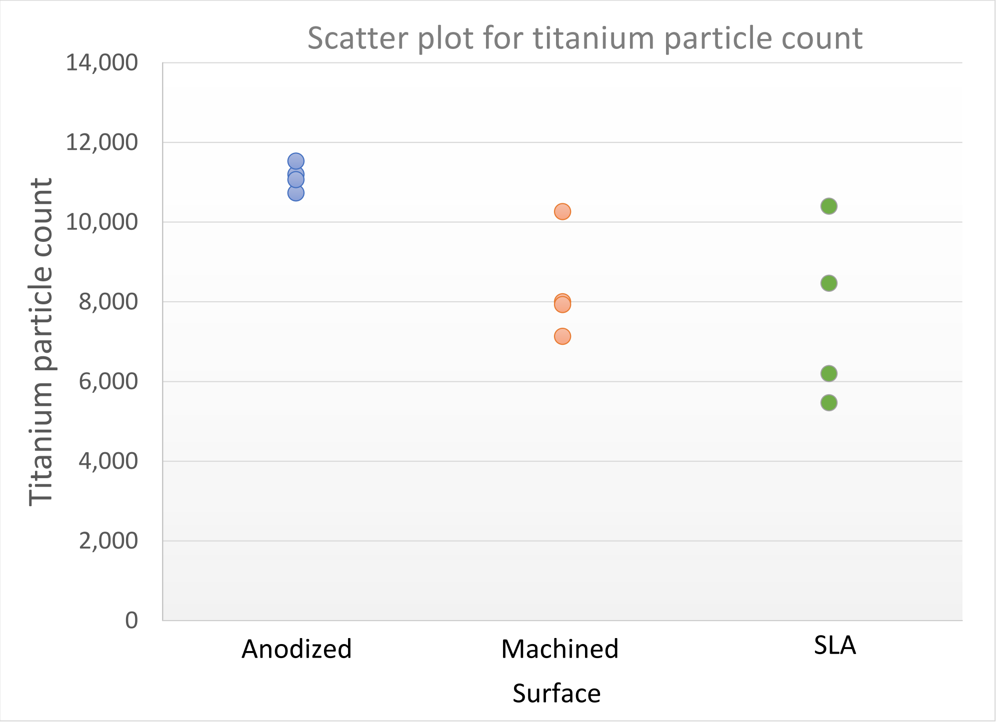

Figure 12. Scatter plot for titanium particle count.

CHAPTER IV

DISCUSSION

While there has not been any direct causal relationship found between the presence of titanium particles and peri-implant mucositis or peri-implantitis, an association can be made regarding the ability of titanium particulate to disrupt cellular function and promote an inflammatory response. This study demonstrated that implant instrumentation by a piezoelectric scaler resulted in titanium particle shedding regardless of the implant surface treatment. However, it became evident that implant surface treatment did play a role in the amount of titanium particle shedding, with more particles being released from the anodized surface. Previous literature has shown a clear difference between implant surfaces when assessed under high magnification (27) (38). It can be speculated that a difference in particle generation between groups may be attributed to the variations in micro surface topography which are produced through different methods of surface modification.

The results of this experiment were found to be similar to findings from other previously reported studies. A study by Pettersson *et al.* found that implants with the anodized surface shed a larger quantity of titanium particulate in the surrounding bone during implant insertion as compared to machined implants (83). Although in the current study no significant difference was noted between anodized and machined implants, a trend towards a significant difference was observed with increased particle shedding from the anodized group as compared to the machined group. Another study by Wu *et al.* compared the titanium particle release from three different implant surfaces during simulated implantoplasty (84). In this experiment, anodized, SLA, and laser etched implants were subjected to instrumentation with a titanium brush designed for implant decontamination. Results from this study also indicated that the SLA surface released a lower titanium particle count as compared to implants with an anodized surface.

Additionally, it is important to mention the finding of titanium particulate in the absence of overt instrumentation i.e., water lavage only. This reaffirms the previous finding from Harrell *et al.* which also revealed the release of titanium particles with the use of water spray only (66). This finding is particularly concerning, in that it shows the ease at which titanium particles may be displaced from an implant surface. It should be noted though that in the current study, titanium particulate count was considerably lower for the active control group as compared to the instrumented implants.

It has been established in the literature that particle size has a biologic impact on cellular responses (76), (77), (85). In the current experiment, a range of particle sizes and shapes were generated as the result of piezoelectric instrumentation. Particle size as small as 2 µm could be identified in the collected samples. This is worrisome since titanium particle size in the 1-10 µm range has been shown to invoke a marked inflammatory response (85), (86).

Future studies on the evaluation of titanium decontamination via instrumentation should investigate minimally abrasive approaches to decontamination in an attempt to avoid titanium particle generation. Kotsakis *et al.* investigated the effects of various mechanical implant cleaning interventions on titanium particle generation (86). It was noted that instrumentation of SLA titanium discs with water jet spray caused little alteration to the titanium while also removing the majority of plaque biofilm. In the current study, the titanium piezoelectric tip caused considerable alterations to the implant body contributing to the significant amount of titanium particle release. Future studies should consider less invasive measures of decontamination such as water jet irrigation.

Implants in this study were never exposed to any corrosive processes prior to instrumentation. When titanium becomes corroded, there is an increased release of metal ions into the surrounding environment which may enhance titanium particulate that is generated (86), (87). Future studies investigating particle release from differing implant surfaces should consider exposing the implants to a microbial environment as this has been shown to induce and replicate corrosive processes that are seen intraorally.

Lastly, the most apparent limitation of this study is the restricted sample size. Although we cannot draw a definitive conclusion regarding increased particle release from anodized implant surfaces, results suggest a distinct difference between dental implant surfaces. Future studies looking at variations in titanium particles generated from differing surface textures should consider a larger sample size to increase validity of the results.

CHAPTER V

CONCLUSION

This *in vitro* study found a significant difference in the amount of titanium particles that were shed between implants with different surface modifications (machined, SLA, anodized) that had undergone instrumentation with a piezoelectric scaler using a titanium tip. This was the first study to date that assessed the particle quantity generated between these three surfaces with this method of instrumentation. Results from this study revealed that the implants with an anodized surface, when instrumented with a titanium piezoelectric tip, released a larger quantity of particles as compared to implants with an SLA surface. This suggests that dental implant surface modification plays a role in the amount of titanium particle shedding.

This study also found that all implants which were instrumented with a titanium tip in a piezoelectric scaler resulted in alterations to the implant surface. This finding places into question whether the benefits of decontamination with titanium piezoelectric tip outweighs the resulting damage to the implant surface. This also places emphasis on a need to explore other methods of decontamination that are less invasive.

Whether these results have a clinically significant impact on the development of peri-implantitis and/or peri-implant mucositis remains to be determined. Currently the body of evidence supporting titanium particulate as an etiologic factor in the development of peri-implant disease is limited. Future studies should be conducted to determine the full impact that titanium particulate plays in the promotion of peri-implantitis and peri-mucositis.

REFERENCES

1. Buser, D., Sennerby, L., and De Bruyn, H. (2017). Modern Implant Dentistry Based on Osseointegration: 50 Years of Progress, Current Trends and Open Questions. *Periodontology 2000*, 73, 7-21.

2. Vasak, C., Fiederer, R., and Watzek, G. (2007). EAO Communication. *Clinical Oral Implants Research*, 18: xiii-xx.

3. Elani, H. W., Starr, J. R., Da Silva, J. D., and Gallucci, G. O. (2018). Trends in Dental Implant Use in the U.S., 1999-2016, and Projections to 2026. *Journal of dental research*, 97(13), 1424–1430.

4. Tang, L., Lund, J. P., Taché, R., Clokie, C. M., & Feine, J. S. (1999). A within-subject comparison of mandibular long-bar and hybrid implant-supported prostheses: evaluation of masticatory function. *Journal of dental research*, 78(9), 1544–1553.

5. Jofre, J., Castiglioni, X., and Lobos, C. A. (2013). Influence of minimally invasive implant-retained overdenture on patients' quality of life: a randomized clinical trial. *Clinical oral implants research*, 24(10), 1173–1177.

6. Hartlev, J., Kohberg, P., Ahlmann, S., Andersen, N. T., Schou, S., and Isidor, F. (2014). Patient satisfaction and esthetic outcome after immediate placement and provisionalization of single-tooth implants involving a definitive individual abutment. *Clinical oral implants research*, 25(11), 1245–1250.

7. Ring, ME (1993). Dentistry: an illustrated history. Abradale Press. 3rd ed.

8. Riaud, X. (2017). History of Dental Implantology. *Austin Journal of Dentistry*, 4(4), 1080.

9. Abraham C. M. (2014). A brief historical perspective on dental implants, their surface coatings and treatments. *The open dentistry journal*, 8, 50–55.

10. Cochran, D. (1996). Implant Therapy I. *Annals of Periodontology*, 1: 707-791.

11. Brånemark, P. I., Adell, R., Breine, U., Hansson, B. O., Lindström, J., and Ohlsson, A. (1969). Intra-osseous anchorage of dental prostheses. I. Experimental studies. *Scandinavian journal of plastic and reconstructive surgery*, 3(2), 81–100.

12. Brånemark, P. I., Hansson, B. O., Adell, R., Breine, U., Lindström, J., Hallén, O., and Ohman, A. (1977). Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scandinavian journal of plastic and reconstructive surgery. Supplementum*, 16, 1–132.

13. Schroeder, A., Sutter, F., Ledermann, P. D., and Stich, H. (1984). ITI--Internationales Team für orale Implantologie. Bisherige Erfahrungen mit dem ITI-Doppelhohlzylinderimplantat Typ K [Current experience with the ITI double hollow cylinder implant type K. Internationales Team für orale Implantologie]. Schweizerische Monatsschrift fur Zahnmedizin = Revue mensuelle suisse d'odonto-stomatologie = Rivista mensile svizzera di odontologia e stomatologia, 94(6), 503–510.

14. Weber HP., Buser D.A., and Weingart D. (2002). The ITI Dental Implant System. In: Greenberg A.M., Prein J. (eds) Craniomaxillofacial Reconstructive and Corrective Bone Surgery. Springer, New York, NY.

15. Sutter, F., Schroeder, A., and Buser, D. A. (1988). The new concept of ITI hollow-cylinder and hollow-screw implants: Part 1. Engineering and design. *The International journal of oral & maxillofacial implants*, 3(3), 161–172.

16. Bothe, R.T., Beaton, K.E., and Davenport, H.A. (1940). "Reaction of bone to multiple metallic implants". *Surg Gynecol Obstet*, 71: 598–602.

17. Leventhal G.S. (1951). Titanium, a metal for surgery. *The Journal of bone and joint surgery. American volume*, 33-A (2), 473–474.

18. Brånemark, P. I. (1961). Experimental Investigation of Microcirculation in Bone Marrow. *Angiology*, 12(7), 293–305.

19. Brånemark P. I. (1983). Osseointegration and its experimental background. *The Journal of prosthetic dentistry*, 50(3), 399–410.

20. Brånemark P. I., Zarb G., and Albrektsson T. (1985). Introduction to osseointegration. In: Tissue integrated Prostheses. *Osseointegration in Clinical Dentistry Quintessence*, 1-76.

21. Wang, Q., Zhou, P., Liu, S., Attarilar, S., Ma, R. L., Zhong, Y., and Wang, L. (2020). Multi-Scale Surface Treatments of Titanium Implants for Rapid Osseointegration: A Review. *Nanomaterials*, 10(6), 1244.

22. Le Guéhennec, L., Soueidan, A., Layrolle, P., and Amouriq, Y. (2007). Surface treatments of titanium dental implants for rapid osseointegration. *Dental materials: official publication of the Academy of Dental Materials*, 23(7), 844–854.

23. Souza, J., Costa Oliveira, B. E., Bertolini, M., Lima, C. V., Retamal-Valdes, B., de Faveri, M., Feres, M., and Barão, V. (2020). Titanium particles and ions favor dysbiosis in oral biofilms. *Journal of periodontal research*, 55(2), 258–266.

24. Nicholson, J. (2020). Titanium Alloys for Dental Implants: A Review. *Prosthesis*, 2(2):100-116.

25. Cordeiro, J. M., Beline, T., Ribeiro, A., Rangel, E. C., da Cruz, N. C., Landers, R., Faverani, L. P., Vaz, L. G., Fais, L., Vicente, F. B., Grandini, C. R., Mathew, M. T., Sukotjo, C., and Barão, V. (2017). Development of binary and ternary titanium alloys for dental implants. *Dental materials: official publication of the Academy of Dental Materials*, 33(11), 1244–1257.

26. Özcan, M., and Hämmerle, C. (2012). Titanium as a Reconstruction and Implant Material in Dentistry: Advantages and Pitfalls. *Materials*, 5(9), 1528–1545.

27. Dohan Ehrenfest, D. M., Vazquez, L., Park, Y. J., Sammartino, G., and Bernard, J. P. (2011). Identification card and codification of the chemical and morphological characteristics of 14 dental implant surfaces. *The Journal of oral implantology*, 37(5), 525–542.

28. Dong, H., Liu, H., Zhou, N., Li, Q., Yang, G., Chen, L., and Mou, Y. (2020). Surface Modified Techniques and Emerging Functional Coating of Dental Implants. *Coatings [Basel]*, 10(11), 1012.

29. Wennerberg, A., Ide-Ektessabi, A., Hatkamata, S., Sawase, T., Johansson, C., Albrektsson, T., Martinelli, A., Södervall, U. and Odelius, H. (2004). Titanium release from implants prepared with different surface roughness. *Clinical Oral Implants Research*, 15: 505-512

30. Wennerberg, A., and Albrektsson, T. (2006). Implant surfaces beyond micron roughness: Experimental and clinical knowledge of surface topography and surface chemistry. *Inter Dent SA*. 8.

31. Cochran, D. L., Buser, D., ten Bruggenkate, C. M., Weingart, D., Taylor, T. M., Bernard, J. P., Peters, F., and Simpson, J. P. (2002). The use of reduced healing times on ITI implants with a sandblasted and acid-etched (SLA) surface: early results from clinical trials on ITI SLA implants. *Clinical oral implants research*, 13(2), 144–153.

32. Coelho, P. G., Jimbo, R., Tovar, N., and Bonfante, E. A. (2015). Osseointegration: hierarchical designing encompassing the macrometer, micrometer, and nanometer length scales. *Dental materials: official publication of the Academy of Dental Materials*, 31(1), 37–52.

33. Mendonça, G., Mendonça, D. B., Simões, L. G., Araújo, A. L., Leite, E. R., Duarte, W. R., Cooper, L. F., and Aragão, F. J. (2009). Nanostructured alumina-coated implant surface: effect on osteoblast-related gene expression and bone-to-implant contact in vivo. *The International journal of oral & maxillofacial implants*, 24(2), 205–215.

34. Buser, D., Schenk, R. K., Steinemann, S., Fiorellini, J. P., Fox, C. H., and Stich, H. (1991). Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. Journal of biomedical materials research, 25(7), 889–902.

35. Boyan, B., Lohmann, C., Dean, D., Sylvia, V., Cochran, D., and Schwartz, Z. (2001). *Annual Review of Materials Research*, 31(1), 357-371

36. Mustafa, K., Silva Lopez, B., Hultenby, K., Wennerberg, A., and Arvidson, K. (1998). Attachment and proliferation of human oral fibroblasts to titanium surfaces blasted with TiO2 particles. A scanning electron microscopic and histomorphometric analysis. *Clinical oral implants research*, 9(3), 195–207.

37. Becker, W., Becker, B. E., Ricci, A., Bahat, O., Rosenberg, E., Rose, L. F., Handelsman, M., and Israelson, H. (2000). A prospective multicenter clinical trial comparing one- and two-stage titanium screw-shaped fixtures with one-stage plasma-sprayed solid-screw fixtures. *Clinical implant dentistry and related research*, 2(3), 159–165

38. Jemat, A., Ghazali, M. J., Razali, M., and Otsuka, Y. (2015). Surface Modifications and Their Effects on Titanium Dental Implants. *BioMed research international*, *2015*, 791725.

39. Al-Nawas, B., and Götz, H. (2003). Three-dimensional topographic and metrologic evaluation of dental implants by confocal laser scanning microscopy. *Clinical implant dentistry and related research*, *5*(3), 176–183.

40. Malmqvist, J. P., and Sennerby, L. (1990). Clinical report on the success of 47 consecutively placed Core-Vent implants followed from 3 months to 4 years. *The International journal of oral & maxillofacial implants*, 5(1), 53–60.

41. Albrektsson T. (1998). Hydroxyapatite-coated implants: a case against their use. Journal of oral and maxillofacial surgery*: official journal of the American Association of Oral and Maxillofacial Surgeons*, 56(11), 1312–1326.

42. Albrektsson, T., and Wennerberg, A. (2019). On osseointegration in relation to implant surfaces. *Clinical implant dentistry and related research*, 21 Suppl 1, 4–7.

43. Lausmaa J. (2001). Mechanical, Thermal, Chemical and Electrochemical Surface Treatment of Titanium. In: Titanium in Medicine. Engineering Materials. Springer, Berlin, Heidelberg.

44. Szmukler-Moncler, S., Testori, T. and Bernard, J.P. (2004). Etched implants: A comparative surface analysis of four implant systems. *J. Biomed. Mater. Res.*, 69B: 46-57.

45. Buser, D., Broggini, N., Wieland, M., Schenk, R. K., Denzer, A. J., Cochran, D. L., Hoffmann, B., Lussi, A., and Steinemann, S. G. (2004). Enhanced bone apposition to a chemically modified SLA titanium surface. *Journal of dental research*, 83(7), 529–533.

46. Buser, D., Mericske-Stern, R., Dula, K., and Lang, N. P. (1999). Clinical experience with one-stage, non-submerged dental implants. *Advances in dental research*, 13, 153

47. Albrektsson, T., Zarb, G., Worthington, P., and Eriksson, A. R. (1986). The long-term efficacy of currently used dental implants: a review and proposed criteria of success. The *International journal of oral & maxillofacial implants*, 1(1), 11–25.

48. Moraschini, V., Poubel, L. A., Ferreira, V. F., and Barboza, E. (2015). Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: a systematic review. *International journal of oral and maxillofacial surgery*, 44(3), 377–388.

49. Howe, M. S., Keys, W., and Richards, D. (2019). Long-term (10-year) dental implant survival: A systematic review and sensitivity meta-analysis. *Journal of dentistry*, 84, 9–21.

50. Wennerberg, A., Albrektsson, T., and Chrcanovic, B. (2018). Long-term clinical outcome of implants with different surface modifications. *European journal of oral implantology*, 11 Suppl 1, S123–S136.

51. Derks, J., and Tomasi, C. (2015). Peri-implant health and disease. A systematic review of current epidemiology. Journal of clinical periodontology, 42 Suppl 16, S158–S171.

52. Lindhe, J., Meyle, J., and Group D of European Workshop on Periodontology (2008). Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. *Journal of clinical periodontology*, 35(8 Suppl), 282–285.

53. Lang, N. P., Berglundh, T., and Working Group 4 of Seventh European Workshop on Periodontology (2011). Peri-implant diseases: where are we now?--Consensus of the Seventh European Workshop on Periodontology. *Journal of clinical periodontology*, 38 Suppl 11, 178–181.

54. Sanz, M., Chapple, I. L., and Working Group 4 of the VIII European Workshop on Periodontology (2012). Clinical research on peri-implant diseases: consensus report of Working Group 4. *Journal of clinical periodontology*, 39 Suppl 12, 202–206.

55. Araujo, M. G., and Lindhe, J. (2018). Peri-implant health. *Journal of clinical periodontology*, *45 Suppl 20*, S230–S236.

56. Berglundh, T., Armitage, G., Araujo, M. G., Avila-Ortiz, G., Blanco, J., Camargo, P. M., Chen, S., Cochran, D., Derks, J., Figuero, E., Hämmerle, C., Heitz-Mayfield, L., Huynh-Ba, G., Iacono, V., Koo, K. T., Lambert, F., McCauley, L., Quirynen, M., Renvert, S., Salvi, G. E., Schwarz, F., Tarnow, D., Tomasi, C., Wang HL., and Zitzmann, N. (2018). Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of clinical periodontology*, *45 Suppl 20*, S286–S291.

57. Heitz-Mayfield, L., and Salvi, G. E. (2018). Peri-implant mucositis. *Journal of clinical periodontology*, *45 Suppl 20*, S237–S245.

58. Schwarz, F., Derks, J., Monje, A., and Wang, H. L. (2018). Peri-implantitis. *Journal of clinical periodontology*, *45 Suppl 20*, S246–S266.

59. Renvert, S., Persson, G. R., Pirih, F. Q., and Camargo, P. M. (2018). Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. *Journal of clinical periodontology*, *45 Suppl 20*, S278–S285.

60. Van Dyke TE, Sheilesh D. Risk factors for periodontitis. *Journal of the International Academy of Periodontology*. 2005 Jan;7(1):3-7.

61. Willis-Owen, C. A., Keene, G. C., and Oakeshott, R. D. (2011). Early metallosis-related

failure after total knee replacement: a report of 15 cases. *The Journal of bone and joint surgery*. British volume, 93(2), 205–209.

62. Bansal, T., Aggarwal, S., Dhillon, M. S., and Patel, S. (2020). Gross trunnion failure in metal on polyethylene total hip arthroplasty-a systematic review of literature. *International orthopaedics*, 44(4), 609–621.

63. Noronha Oliveira M, Schunemann WVH, Mathew MT, Henriques B, Magini RS, Teughels W, Souza JCM. Can degradation products released from dental implants affect peri-implant tissues*? J Periodontal Res*, 2018 Feb;53(1):1-11.

64. Donaldson, J. R., Miles, J., Sri-Ram, K., Poullis, C., Muirhead-Allwood, S., & Skinner, J. (2010). The Relationship between the Presence of Metallosis and Massive Infection in Metal-on-Metal Hip Replacements. *HIP International*, *20*(2), 242–247.

65. Wilson T. G., Jr (2021). Bone loss around implants-is it metallosis? *Journal of periodontology*, *92*(2), 181–185.

66. Harrel, S. K., Wilson, T. G., Jr, Pandya, M., and Diekwisch, T. (2019). Titanium particles generated during ultrasonic scaling of implants. *Journal of periodontology*, 90(3), 241–246.

67. Mints, D., Elias, C., Funkenbusch, P., and Meirelles, L. (2014). Integrity of implant surface modifications after insertion. *The International journal of oral & maxillofacial implants*, 29(1), 97–104.

68. Delgado-Ruiz, R., & Romanos, G. (2018). Potential Causes of Titanium Particle and Ion Release in Implant Dentistry: A Systematic Review. *International journal of molecular sciences*, 19(11), 3585.

69. Wilson, T. G., Jr, Valderrama, P., Burbano, M., Blansett, J., Levine, R., Kessler, H., & Rodrigues, D. C. (2015). Foreign bodies associated with peri-implantitis human biopsies. *Journal of periodontology*, *86*(1), 9–15.

70. Grande, F., & Tucci, P. (2016). Titanium Dioxide Nanoparticles: a Risk for Human Health? Mini reviews in medicinal chemistry, 16(9), 762–769.

71. Weingart, D., Steinemann, S., Schilli, W., Strub, J. R., Hellerich, U., Assenmacher, J., and Simpson, J. (1994). Titanium deposition in regional lymph nodes after insertion of titanium screw implants in maxillofacial region. International journal of oral and maxillofacial surgery, 23(6 Pt 2), 450–452.

72. He, X., Reichl, F. X., Wang, Y., Michalke, B., Milz, S., Yang, Y., Stolper, P., Lindemaier, G., Graw, M., Hickel, R., and Högg, C. (2016). Analysis of titanium and other metals in human jawbones with dental implants - A case series study. Dental materials: official publication of the Academy of Dental Materials, 32(8), 1042–1051.

73. Meyer, U., Bühner, M., Büchter, A., Kruse-Lösler, B., Stamm, T., and Wiesmann, H. P. (2006). Fast element mapping of titanium wear around implants of different surface structures. Clinical oral implants research, 17(2), 206–211.

74. Pioletti, D. P., Takei, H., Kwon, S. Y., Wood, D., and Sung, K. L. (1999). The cytotoxic effect of titanium particles phagocytosed by osteoblasts. Journal of biomedical materials research, 46(3), 399–407.

75. Wachi, T., Shuto, T., Shinohara, Y., Matono, Y., and Makihira, S. (2015). Release of titanium ions from an implant surface and their effect on cytokine production related to alveolar bone resorption. Toxicology, 327, 1–9.

76. Choi, M. G., Koh, H. S., Kluess, D., O'Connor, D., Mathur, A., Truskey, G. A., Rubin, J., Zhou, D. X., and Sung, K. L. (2005). Effects of titanium particle size on osteoblast functions in vitro and in vivo. Proceedings of the National Academy of Sciences of the United States of America, 102(12), 4578–4583.

77. Kumazawa, R., Watari, F., Takashi, N., Tanimura, Y., Uo, M., and Totsuka, Y. (2002). Effects of Ti ions and particles on neutrophil function and morphology. Biomaterials, 23(17), 3757–3764.

78. Wei, X., Zhang, X., Zuscik, M. J., Drissi, M. H., Schwarz, E. M., and O'Keefe, R. J. (2005). Fibroblasts express RANKL and support osteoclastogenesis in a COX-2-dependent manner after stimulation with titanium particles. Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research, 20(7), 1136–1148.

79. Irshad, M., Scheres, N., Crielaard, W., Loos, B. G., Wismeijer, D., and Laine, M. L. (2013). Influence of titanium on in vitro fibroblast-Porphyromonas gingivalis interaction in peri-implantitis. Journal of clinical periodontology, 40(9), 841–849.

80. Fretwurst, T., Buzanich, G., Nahles, S., Woelber, J. P., Riesemeier, H., and Nelson, K. (2016). Metal elements in tissue with dental peri-implantitis: a pilot study. Clinical oral implants research, 27(9), 1178–1186.

81. Olmedo, D. G., Nalli, G., Verdú, S., Paparella, M. L., and Cabrini, R. L. (2013). Exfoliative cytology and titanium dental implants: a pilot study. Journal of periodontology, 84(1), 78–83.

82. Safioti, L. M., Kotsakis, G. A., Pozhitkov, A. E., Chung, W. O., and Daubert, D. M. (2017). Increased Levels of Dissolved Titanium Are Associated with Peri-Implantitis - A Cross-Sectional Study. Journal of periodontology, 88(5), 436–442.

83. Pettersson, M., Pettersson, J., Molin Thorén, M., and Johansson, A. (2017). Release of titanium after insertion of dental implants with different surface characteristics - an ex vivo animal study. Acta biomaterialia odontologica Scandinavica, 3(1), 63–73.

84. Wu, X., Cai, C., Koticha, T., and Suárez López del Amo, F. (2020). Comparison of Debris Particle Release After Decontamination Therapy Among Multiple Implant Systems. SWSP Probe, 20.

85. Eger, M., Hiram-Bab, S., Liron, T., Sterer, N., Carmi, Y., Kohavi, D., and Gabet, Y. (2018). Mechanism and Prevention of Titanium Particle-Induced Inflammation and Osteolysis. *Frontiers in immunology*, *9*, 2963.

86. Kotsakis, G. A., Black, R., Kum, J., Berbel, L., Sadr, A., Karoussis, I., Simopoulou, M., and Daubert, D. (2020). Effect of implant cleaning on titanium particle dissolution and cytocompatibility. *Journal of periodontology*, 10.1002/JPER.20-0186.

87. Noumbissi, S., Scarano, A., and Gupta, S. (2019). A Literature Review Study on Atomic Ions Dissolution of Titanium and Its Alloys in Implant Dentistry. *Materials (Basel, Switzerland)*, *12*(3), 368.