Curriculum Vitae

Navpreet K. Khatra

Navi.khatra@gmail.com

EDUCATION

Certificate in Prosthodontics, University of Maryland, Baltimore (anticipated June 2018)

Master of Science in Biomedical Science, University of Maryland, Baltimore (anticipated June 2018)

Doctor of Dental Surgery, NYU College of Dentistry NY, New York

Bachelor of Dental Surgery, Luxmi Bai Institute of Dental Sciences & Hospital Patiala, India

WORK EXPERIENCE

Clinical Instructor, University of Maryland Baltimore, January 2018 to present

Community service, Medical and Immigration camp for newly arrived immigrants through local temple (Panja organization), March 2017

Community service in Odyssey House (for drug and alcohol abuse), Harlem, NY, September 2014- March 2015 (12/ 350 students)

Attended various dental camps in New York, including Henry Schein outreach and Bright Smiles, Bright Futures Program, July 2013, 2014

Dental assistant at Bhullar Dental Clinic, Fort Washington, Maryland, May 2010 – October 2011 Fort Washington, Maryland

Dentist, Singla Dental Clinic, Punjab, India, January 2009 – January 2010

Dentist (Intern) Luxmi Bai Institute of Dental Sciences and Hospital, Punjab, India, January 2008 – December 2008

Fundraising officer for local community outreach, Punjab, India, May 2007- 2008

Volunteer, Smile India, Volunteer dental work for underserved communities in Punjab, India, March 2007, 2006

HONORS AND AWARDS

- Honors in Prosthodontics, NYU (5/360 students), 2014- 2015
- Honors in Clinical Dentistry, LBDC 2008- 2009
- Selected as one of the top students in patient communication skills, NYU, 2015
- Winner of National Scholarship, and Hinduja Scholarship, 2001
- Awarded with ‘College Color’ which stands for the most helpful senior to other students, 2008
- Clinical student coordinator, NYU 2013- 2015
- Student Liaison Officer, LBDC student association 2008- 2009

- Member of Clinical Dental Assistant Supervisor selection committee, Department of Advanced Oral Sciences and Therapeutics, UMSOD 2017

- Member “Women of Color in Dentistry” and “Advanced Placement Students Dental Association” (APSDA); focuses on social and dental services, NYU 2013-2015

PRESENTATIONS

- Poster presentation: ACP meeting 2017, Bacterial Adhesion To Various Implant Surfaces

- Poster presentation: AAFP meeting 2017, Digital Workflow Of A PFM Crown To An Implant Crown

- Presenter: Oral Presentation at Robert Schoor Northeast Implant Symposium 2017, Digital Planning and Execution of Implant Supported Full Arch Restoration

- Poster presentation: ACP meeting 2016, Technique To Increase The Retention Of Mandibular Denture

SKILLS

- Language: Proficient in Punjabi, Hindi and English

- An overall score of 107/120 in TOEFL. Certification in November 2011

ABSTRACT

Title of Thesis: Bacterial Adhesion to Various Implant Surfaces

Navpreet K. Khatra, Master of Science, 2018

Thesis Directed by: Radi Masri

Dental implants provide a major course of treatment for patients who are partially or completely edentulous. These implants are biocompatible metal anchors that are surgically positioned in the jawbone to support the prostheses where natural teeth are missing. However, the use of dental implants has some disadvantages, which can result in complications. Once the clean implant surface is exposed in the oral cavity, it is immediately coated with salivary pellicle and subsequently colonized by oral microbial species. In fact, microbial adhesion and accumulation on implants are considered to play major roles in the pathogenesis of peri-implant mucositis and peri-implantitis. The physico- chemical characteristics of specific material surface are known to significantly influence the bacterial adhesion process. Therefore, the surface characteristics of dental implants have been refined and restructured over a period of time to improve the interaction of implants with host cells and tissues. Hence, investigating the microbiological aspects related to implant surfaces will provide important insights relevant to expectations of treatment outcome. To that end, in this study, we aimed to comparatively evaluate microbial adherence and accumulation on five different types of implants. Specimens were provided by Dental Implant Systems, Biodenta Group and

implant surfaces included: .3- .5 μm anodized surface, 1 μm BST surface, .8- 1.0 μm anodized surface, 1.6μm SLA surface and .3- .4 μm machined surface. To assess microbial adherence, the cariogenic bacterial species *Streptococcus mutans* and the bacterial pathogen *Staphylococcus aureus* were studied. Results from these studies were analyzed using analysis of variance (ANOVA) and significant differences were further analyzed by Tukey's Honestly Significant Difference (HSD) test. Pearson's r was also used to evaluate the association between surface roughness and bacterial accumulation. The present study has demonstrated that not only surface roughness but other physicochemical properties such as surface charge, energy, wettability and biological factors such as host immune response and oral hygiene influence bacterial adhesion and accumulation around implant surfaces.

Bacterial Adhesion to Various Implant Surfaces

by
Navpreet K. Khatra

Thesis submitted to the Faculty of the Graduate School of the
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Navpreet K Khatra, DDS

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Introduction

Implants

The goal of modern dentistry is to restore the patient's normal oral functions including speech as well as aesthetics, regardless of the disease condition or extent of injury to the stomatognathic system. Dental implants serve as an ideal option for people who are partially or completely edentulous due to periodontal disease, injury, or other pathological reason.[1] The history of dental implants is a rich and fascinating travelogue through time and a range of implant materials have been developed and used including gold ligature wire, shells, ivory to chromium, cobalt, iridium and platinum. In addition to the various materials, numerous structures and designs were also generated to replace the positions that natural teeth once held such as spiral stainless steel implant designs to double helical creations and endosseous root forms.[2] Additionally, investigating surface treatment methods to decrease the healing time for osseointegration has also been a focus of much research in the field of biomaterials. As research advances, materials, forms, and surface coatings are constantly refined and restructured in order to expand on the tooth replacement options currently available for clinical applications.[3] Impressively, studies have shown a success rate of over 90% for dental implants in various clinical situations.[4] Nevertheless, physicochemical characteristics such as surface charge, energy, and composition can be continually manipulated to further improve on the interaction of implants with host cells and tissues.[5]

Surface Charge

The surface energy of a biomaterial is determined by the material's surface-charge density and the net polarity of the charge; compared to an electrically neutral surface, a surface with net positive or negative charge may be more hydrophilic. In fact, the surface-charge of a dental implant is known to be a key factor that guides bone cell adhesion and early stage bone mineralization in the bone-implant interface.[6] According to C Y Guo et al. a successful hydroxyapatite inducer for titanium implants could be a material that develops both negative surface-charge and abundant OH- groups in physiologically related fluids to serve for bone-bonding materials. This process would allow osteoblasts to more actively proliferate on a negatively charged biomaterial surface. In contrast, cell adhesion and proliferation on positively charged biomaterial were found to be subdued. For example, Ca^{2+} ions have superior binding affinity to a negatively charged biomaterial surface. Further, these ions affect crystal nucleation and positively attract cell-adhesion proteins (e.g., integrins, fibronectin, and osteonectin), which significantly impact attachment, adhesion, and spreading of osteoblasts. Consequently, osteoblasts attach and proliferate on a matrix grown on the bone-like apatite layer formed with Ca^{2+} ions, which may result in faster and stronger bone-to-implant bonding. In contrast, a positively charged implant surface attracts anionic groups, which act as antiadhesive molecules, thereby negatively affecting osteoblast adhesion.[7] Hence, a charged implant surface can induce electrical attraction or repulsion between the implant's surface and the surrounding chemical species, depending on their polarity.[8]

Surface Energy

The implant surface energy is an important factor in regulating osteogenesis, which is assessed by contact angle measurement i.e. the wettability of a surface. Pure titanium surfaces exhibit high surface energy due to the oxide layer that grows spontaneously at the room temperature.[9] Higher surface energy has been hypothesized to be desirable for implants as it enhances interaction between the implant surface and biologic environment. This truly integrated surface can carry more loads than the adjacent tissue elements.[10] On the other hand, low surface energy implant materials are characterized by fibrous capsule interface between the implant and the bone. Such surfaces, at the time of insertion retain these poor adhesive layers for a longer period of time.[11]

Surface Composition

The chemical composition of the implant surface often differs from that of the bulk material due to preparation methods and impurities trapped in the implant surface. The surface of a dental implant is the only part that is in contact with the bio-environment and the uniqueness of the surface directs the response and affects the mechanical strength of the implant/tissue interface. Cell attachment, adhesion, and spreading are the first phase of the interaction between the osteogenic cells and the implant. These reactions affect the cell's capacity to adhere and proliferate on the implant's surface and subsequently generate bone tissue surrounding the implant. Cell-implant interactions

depend upon the implant's surface topography, chemistry and surface energy. The surface layer contains reactive bonds, a continuous exchange of water and various ions influencing the binding of proteins to the surface and subsequent cell reaction.[12]

The surface treatment layer on the implant is required to increase the functional surface area of the implant-bone interface, in order to enhance adherence of platelets and fibrin to implant surface, improve primary stability, and expedite bone deposition and osseointegration. Surface treatment of dental implants may include mechanical treatments (machining and grit blasting), chemical treatments (acid etching, plasma treatment[13]), electrochemical treatments (anodic oxidation), vacuum treatments, thermal treatments, and laser treatments. [14]These treatments are designed to manipulate surface roughness, surface charge, surface energy, and surface composition to affect the interaction of implants with cells and tissues and ultimately affect the success of dental implants.[15]

Implant surfaces with varying surface chemistry, surface topography and roughness are commercially available. Smooth implants have Sa value lower than 0.5 μm whereas minimally rough implants have a roughness (Sa) of between 0.5 to 1.0 μm and are represented by turned NobelBiocare (Branemark) implants and by acid etched implants for example.[16] Moderately roughened surfaces vary between 1.0 and 2 .0 μm and include almost all modern implants, such as the Astra Tech TiOblast TM and OsseoSpeed TM surfaces, Nobel Biocare TiUnite, Straumann SLA and Dentsply Cellplus designs. In contrast, rough implants are those with Sa above 2.0 μm and are exemplified

by plasma sprayed devices and an example of such an implant is the Dentsply Frialit implant.[17]

Titanium

Titanium is a biocompatible metal with low molecular weight, low modulus of elasticity, and excellent corrosion resistance[18] and is widely used in the manufacture of dental implants in the form of commercially pure titanium (cpTi) or an alloy. There are four grades of cpTi, and two titanium alloys that are commonly used in dentistry. Tensile strength and yield strength increase from grade I to grade IV cpTi; titanium alloys are Ti-6AL-4V and Ti-6Al-4V ELI (extra low interstitial). Out of these two alloys, Ti-6AL-4V has high tensile strength and yield strength. All forms of cpTi and Ti alloys have almost the same modulus of elasticity, however, a lower modulus is desirable because it can better transmit forces to the bone.[19] Studies have shown that cpTi is more favorable for osteoblastic differentiation[20] whereas other studies suggest that titanium alloy is much stronger. Titanium alloy decreases the complications of components, implant body fracture or wearing of antirotational features of abutment, which increases the risk of screw loosening.[21] When cpTi and Ti alloy are exposed to air, a layer of Titanium oxide is formed, which plays an important role in corrosion resistance, biocompatibility and osseointegration.

Titanium is dimorphic and exists in two phases; at temperatures up to 882°C, pure titanium exists as a hexagonal close-packed atomic structure (alpha – phase) whereas

above that temperature, the structure is body-centered cubic (beta phase). Ti is brittle when cold, and malleable when hot, however, it can be ductile only when it is free of oxygen and traces of nitrogen or oxygen increase its strength. Ti forms alloys with Al, Cr, Co, Cu, Fe, V, Fe, Ni and Sn[22]; in titanium alloys, aluminum is an alpha phase stabilizer, which increases the strength and decreases the weight of the alloy, whereas, vanadium (V) stabilizes the beta phase. As Al or V is added to Ti, the temperature at which alpha to beta transformation occur changes to a range of temperatures and in these ranges, alpha and beta forms coexist.[23]

Oxide Coating

When metals are exposed to atmosphere, metals oxide layers are formed. Nature of this oxide layer depends on the metal and the conditions under which it was oxidized. The oxide layer, and not the metal itself, determines the chemical properties and the interfacial chemistry. During the implant machining process, fresh cut titanium is exposed to the atmosphere and an oxide layer of greater than 10A is formed in less than a millisecond, and within a minute the oxide thickness will be of the order of 50-100A.[24] Ti forms various stable oxides TiO, TiO_2, Ti_2O_3 with TiO_2 being the most common one.[25]

When an implant is introduced into the body, complex reactions occur at the oxide and the bio-environment interphase where the oxide layer grows, as ions diffuse outward from the metal and inward from the environment. Therefore, the oxide layer that forms in the body is different than the one that forms in the air. CpTi and Ti alloys are

easily passivated to form a stable TiO_2 layer that makes it corrosion resistant.[26] However, overtime changes can be seen on the surface of the metal implant and accumulation of titanium in surrounding tissues is observed. Inert surface oxide film makes titanium highly biocompatible material, with low surface ion release from its surface into the surrounding environment. Importantly, the human body does not reject this surface that forms.[27]

Biocompatibility

There are certain criteria for any potential metallic material to have excellent corrosion resistance; these include (1) ease of oxidation (2) strong adherence of formed oxide to the substrate, (3) density of oxide layer, and (4) protectiveness of formed oxide layer. Titanium is a highly reactive metal and will react within microseconds to form an oxide layer when exposed to the atmosphere as described above. Because of this oxide layer, titanium is stable, even in a biological system including chemical and mechanical environments.[28] During implantation, titanium releases corrosion products (mainly titanium oxide or titanium hydro-oxide) into the surrounding tissue and fluids even though a thermodynamically stable oxide film covers it. An increase in oxide thickness, as well as incorporation of elements from the extra- cellular fluid (P, Ca, and S) into the oxide, changes the oxide stoichiometry, composition, and thickness. The titanium-passivating layer not only produces good corrosion resistance, it also allows

physiological fluids, proteins, and hard and soft tissue to come close and deposit on it directly.[29]

Corrosion Resistance

Rate of corrosion is significantly reduced in the presence of protective surface film. However, stresses can induce mechanical and environmental effects on a metal, changing its properties and the properties of the surface oxide. In these instances, stress may occur in combination with time, temperature and corrosive environment.[30] Presence of aluminum in higher than 6% concentration makes Ti alloy susceptible to stress, corrosion, cracking, and corrosion fatigue through formation of $TiAl_3$. The presence of Vanadium reduces the susceptibility for corrosion cracking due to suppression of formation of $TiAl_3$ compounds.[31] Ti alloys are extremely resistant to corrosion fatigue, making titanium alloy the metal of choice when high corrosion fatigue strength is desired. In some instances, localized corrosion is seen in the metal surface due to the incorporation of contaminants introduced during casting, milling, machining processing etc. Such areas are susceptible to breakdown of the oxide layer, leading to crevice and pitting corrosion. This type of corrosion is rare in Ti alloys.[32]

Turned Surfaces

Turned surfaces are machined or smooth surfaces that were used in the past. On a microscopic level, turned surfaces are not actually smooth, as grooves and ridges are

present on their surface. Presently, turned surfaces are considered to have minimally rough surfaces with Sa 0.5- 1.0 μ.[33] Morphology of this surface plays an important role in cellular behavior and distant osteogenesis is an important characteristic. Interaction of turned surfaces with culture media and serum directly effects the attachment, proliferation and differentiation of osteoblasts. This interaction also influences the production of local cell regulators such as transforming growth factor B and prostaglandin E_2.[34] In a number of studies, success rates of turned surfaces were found to be similar to those of rough surfaces[35] whereas other studies are suggestive of significantly higher success rates of rough surfaces compared to turned surfaces.[36],[37] In a 5 year randomized controlled trial, a significantly higher failure rate was observed in the preload of the turned surfaces as opposed to dual acid etched surfaces with a survival rate of 96.8% for the dual acid etched surface and 86.8% for the turned surface. In low-density bone (type IV bone), survival rates of rough surface implants were found to be 97.1% and turned surfaces 91.6%.[38] In a 3 year randomized controlled trial in patients with periodontitis, Nicu E A et al. found comparable clinical, microbiological and bone biochemical parameters with turned implants.[39] In fact, several clinical studies highlighted an increased risk of peri-implantitis when using rough implant surfaces as compared to turned surfaces.[40],[41]

Sandblasted Surfaces

The aim of the surface sandblasting method is to increase implant surface roughness by using gritting agents such as aluminum oxide and titanium oxide. Surface

roughness depends on the number and speed of the rotations, to which the implant is subjected to, as well as the size and pressure of the particles used. However, there is a large variability in surface appearance in using the sand blasting technique. Studies suggest that sandblasting improves adhesion, proliferation and differentiation of osteoblasts and reduces fibroblast adhesion and proliferation.[42,43,44] Additionally, some studies suggest that BIC (bone implant contact) and removal torque value are better with sandblasted surfaces whether alumina or titanium oxide is used as compared to turned surfaces.[45],[46] A study by Mueller et al. evaluated the interface between bone and titanium implants blasted with Al_2O_3 particles of different sizes (25 μm , 75 u μm and 250 u μm) after 12 weeks of placement and findings from the study demonstrated that all sandblasted surfaces exhibited more bone in contact with the implant surface compared with turned surfaces.[47] Moreover, a high percentage of bone was found in close contact with the implant surface in the sites where 75 μm particles were used.[48] Gotfredsen et al. analyzed removal torque of dental implants with titanium blasted surface (25 μm, Ra=0.88 μm), and turned surface (Ra-0.39 μm). Results from the study demonstrated that following 12 weeks of insertion, TiO_2 blasted implants exhibited significantly greater removal torque force (35.4 N-cm) than turned implants (29.2 N-cm). However, BIC was not significantly different between the two types.[49]

Although blasting has several advantages, there is evidence of adverse effects, such as surface contamination (depending on type of blasting media) and distortion of blasted work piece (depending on blasting manner and intensity).[50] Miyakawa et al. evaluated the surface contamination of abraded titanium and found that despite low

grinding speeds and water-cooling, the abraded surfaces were contaminated by abrasive constituent elements. It was reported that the contamination of titanium is related to its reactivity, as well as its hardness and it negatively influences titanium's resistance to corrosion and its biocompatibility.[51] In 2006, Marinucci et al. conducted a study on three titanium surfaces: machined titanium, microsandblasted titanium (0.5 μm) and macro sandblasted titanium (3 μm). The goal of the study was to investigate the effect of varying surface roughnesses of titanium implant material, on cell proliferation and mRNA expression of specific markers of osteoblast phenotypes. Based on the findings, the authors concluded that macro-sandblasted surfaces are more favorable than micro-sandblasted surfaces for osteoblast differentiation *in vitro.*[52]

Acid-Etched Surfaces

Acid etching is a process that can be accomplished using hydrochloric acid, hydrofluoric acid, sulfuric acid, nitric acid or a combination of any of these acids. The initial surface roughness, type and duration of acid used, and bath temperature all affect the acid etching process.[53] According to some researchers, BIC and resistance to torque removal is much higher for acid etched surfaces compared to turned or sand blasted surfaces. However, dual acid etching produces a micro textured surface, which has significantly better properties. In one study where the surface was roughened using hydrochloric acid and sulfuric acid, surface formed was characterized by an even distribution of very small peaks and valleys and the resistance to torque was four times

greater than turned ones. Similarly, Degidi et al. found that BIC was significantly higher for dual acid etched implants (62.9%) as compared to turned sites (39.5%).[54]

Sandblasted and Acid Etched Surfaces (SLA)

These are modified surfaces produced by blasting and acid etching. Blasting is usually performed with small particles like TiO_2, SiO_2, Al_2O_3 etc. that produces macro texture on the surface. Afterwards, acid- etching is performed on the same surface with acids such as HCl, H_2SO_4 that produce a final micro texture This resultant surface exhibits uniformly scattered gaps and holes and appears to be less rough than plasma sprayed surfaces. Using miniature pigs, Buser et al. conducted a study to test the shear strength of titanium implants sandblasted with 0.25- 0.50 um particles, and etched with HCl or H_2SO_4. The findings demonstrated significant differences between sandblasted and acid-etched (removal torque= 1.43 N-cm) and turned implants (removal torque= 0.26N-cm). However, differences between sandblasted and acid etched as compared to plasma sprayed (removal torque= 1.54 N-cm) were not significantly different.[55] Monclear et al. suggested that the depth and distribution of irregularities, the cavity morphology and the presence of contaminating elements derived from the treatment procedure play an important role in cell behavior.[56] In another study, Carr et al. evaluated the pattern of bone formation in blasted acid etched (Sa= 2.29 μm) and turned surface (Sa=0.35 μm) and found that after 12 weeks of healing, BIC values in sandblasted and acid etched sites were significantly greater than in turned sites. BIC for sand blasted and acid etched surfaces was 60% and for turned surfaces was 40% respectively.[57] H. Climent et al.

evaluated the BIC three months after implant placement, three months after loading and 12 months after loading in dogs. In this study, acid etching was done using HCl and H_2SO_4 and sand blasting with 250-500 μm corundum particles. Findings demonstrated that sand blasted and acid etched surfaces showed significantly higher percentage of BIC (72.33%) as compared to plasma sprayed implants (52.15%) but no differences were observed after a healing period of six months. Following 12 months of loading, BIC values were significantly higher in sand blasted acid etched surfaces (71.68%) than in plasma sprayed surfaces (58.88%).[58] Additionally, sandblasted and acid etched surfaces also presented with better osteoconductive properties and a higher capability to induce cell proliferation than plasma- sprayed surfaces. Smeets et al. in a 10-year retrospective study on success and survival rates of SLA surfaces showed a success rate of 97% and a survival rate of 95.1%.[59]

Plasma Sprayed Surfaces

Plasma sprayed surfaces are prepared by spraying molten metal on a titanium base, which results in a surface with irregularly sized pores and crevices. These properties increase the surface area microscopically by 6-10 times and actual load bearing functional area by 25% to 30%. In addition, this porous surface (Ra= 150-400 μm) increases the tensile strength of the bone implant interface, resists shear forces and improves load transfer and is similar to a three-dimensional surface, which may stimulate adhesion osteogenesis.[60] Therefore, this topography may improve the osseointegration of implants via growth of bone into the coating, forming a mechanical interlock. A study by

Klokkevold et al. evaluated the removal torque in three different implant surfaces: acid etched, plasma sprayed and turned after a healing period of three months and found significant differences between acid etched and turned surfaces and plasma sprayed and turned surfaces. However, the differences between acid etched and plasma sprayed were not statistically different.[61] The disadvantage of plasma sprayed surfaces is the detachment of titanium that happens after implant insertion, which could be related to the friction between the implant surface and the bone during implant placement, which may ultimately lead to implant failure.[62]

Anodized Surfaces

Anodized surfaces are prepared by applying a voltage on the titanium specimen immersed in an electrolyte.[63] This oxidation process changes the characteristics of the oxide layer and improves surface biocompatibility.

The resultant surface presents micro pores of variable diameters, demonstrates lack of cytotoxicity, and enhanced cell attachment and proliferation as compared to turned surfaces. In a study by Ivanoff et al., anodized (Sa=1.17 μm) and turned (Sa=0.78 μm) surfaced micro implants (2.3 x 5 mm) were inserted in human subjects. Following a healing period of 6.6 months, the BIC was found to be significantly higher in anodized (34%) than in turned (13%) implants.[64] These differences may be due to the thicker oxide layer, which increases surface roughness, and changes the surface morphology in terms of porosity and crystal structure.[65] Using a rabbit model, Burgos et al. compared the BIC of implant surface between anodic oxidation and turned surfaces and showed the BIC

values to be 20% (after 7 days), 23% (after 14 days), and 46% (after 28 days) around the oxidized surfaces and 15% (after 7 days), 11% (after 14 days), and 26% (after 28 days) around the machined surfaces. It was concluded from these findings that the moderately rough oxidized surfaces follows a different pattern of osseointegration.[66] In contrast, Huang et al., evaluated the oxidized implant surfaces placed in the posterior maxilla of monkeys and after 16 weeks, the mean BIC was found to be 74%. The authors from that study suggested that this oxidized surface detains a considerable osteoconductive potential promoting a high level of implant osseointegration in type IV bone in the posterior maxilla.[67]

Hydroxyapatite Coating (HA)

The bond between bone and HA coating and strength of HA to bone interface is greater than that of titanium to bone and even greater than TPS to bone. The space between the implant and the bone may affect the percentage of bone contact after healing and gap healing may be enhanced by the HA coating which has a surface roughness of 0.7 μm and thickness of 50-70μm. These surfaces also provide better implant anchorage as osteoblasts respond to this contact between the bone and the implant through immediate changes in gene expression, proliferation, differentiation and extracellular matrix synthesis.[68] Several studies were done on apatite-like formation without HA coating. According to Pattanayak et al., Ti can form a bone-like apatite layer on its surface in simulated body fluid (SBF) when it is treated in NaOH. When pre-treated titanium is exposed to SBF, the alkali ions are released from the surface into the

surrounding fluid. The sodium ions increase the degree of supersaturation of the soaking solution with respect to apatite by increasing pH.[69] On the other hand, the released Na^+ causes an increase in external alkalinity that triggers an inflammatory response leading to cell death. Furthermore, it was found that the rate of apatite formation was not significantly influenced by a lower amount of Na^+ ion in the surface layer, and Ti with the lowest content of Na^+ could be more suitable for implantation in the human body.[70] Generally, titanium is covered with a passive oxide layer, however in NaOH, this passive film dissolves and an amorphous layer containing alkali ions is formed. When exposed to SBF, the alkali ions are released from the amorphous layer and hydronium ions center into the surface layer, resulting in the formation of Ti-OH groups in the surface. The acid etching of Ti in HCl under inert atmosphere leads to the formation of a micro-roughened surface, which keeps its integrity after alkali treatment in NaOH. It was shown that the apatite nucleation was uniform and the thickness or precipitated hydroxycarbonated apatite layer increased continuously with time.[71]

Coated surfaces are also expensive in comparison to uncoated surfaces. Mimura et al. characterized morphologically and chemically coated-substrate interface of a commercially available dental implant coated with plasma-sprayed HA, when subjected to mechanical environment. A thin Ti oxide film containing Ca and P was found at the interface on Ti-6Al-4V and when the implant was subjected to mechanical stress, a mixed mode of cohesive and interfacial fractures occurred. The cohesive fracture was due to separation of the oxide film from the substrate, while the interfacial fracture was due to the exfoliation of the coating from the oxide film bonded to the substrate.[72] Yoon et al.

reported two accidentally failed HA coated implants which were retrieved after 18 months of loading, and found that dense bone was formed in close proximity to implant surface and interfaces of each implant were filled with bone.[73] In a study by Schwartz et al., high survival rates for HA coated implants were found following 3, 8, and 12 years follow up with a 93.2% survival rate for HA implant which was significantly higher than that of titanium implants (89%).[74]

Oskouei et al. compared the fatigue resistance of HA blasted on Ti-6Al-4V with film thickness ranging from 0, 25, 50, 75, 100 and 150 μm. The combined findings from the study demonstrated that (i) samples with 150 μm have significantly decreased fatigue resistance, and (ii) HA coatings with 25- 50 μm had no observable delamination during fatigue tests, while coatings with 75- 150 μm thick spalled following but not prior to the initiation of the first fatigue crack in the substrate.[75] The disadvantage of HA coated surfaces is the risk of cracking, flaking or scaling on insertion, especially when they are inserted into dense bone. Moreover, increased surface roughness increases the risk of plaque retention and bacterial contamination.[76]

Zirconia

With the advancements in biomaterials science and industrial technology, interest in ceramics for dental application has been renewed. Ceramics, particularly the yttrium-stabilized tetragonal polycrystalline zirconia (Y-TZP), exhibit improved mechanical properties, which make them suitable substrates for the fabrication of dental implants.[77]

Zirconia is a biocompatible and esthetic material with mechanical properties that are better than Alumina, as it is highly resistant to corrosion, flexion and fracture. Yttria stabilized tetragonal zirconia polycrystalline (Y-TZP) materials exhibits superior corrosion and wear resistance, as well as a high flexural strength (800 to 1000 MPa) compared to other dental ceramics.[78] Contact with bone and soft tissue is similar to that observed in Titanium and therefore, zirconia can be used to produce entire implant surfaces or as a coating. In an *in vitro* study, Osman et al. reported the fracture strength of one-piece unloaded zirconia implants to be 512.9 N versus 410.7 N after artificial loading.[79] However in some studies on one piece and two-piece zirconia implants, decrease in fractural strength resistance was found following cyclic loading and implant preparations, questioning clinical use of zirconia implants.[80] According to Smeets et al., the amount of bone formed one and six months after implant placement did not differ between titanium and zirconia implants.[81] Using a rabbit model, Sennerby et al. four weeks after implantation, found the BIC of zirconia to be 68.4% with absence of epithelial down growth, foreign bone reaction or fibrous tissue between the bone and the implant.[82]

Temperature Effect

At ambient pressure, unalloyed zirconia can assume three crystallographic forms, depending on the temperature. At room temperature and upon heating to 1170°C, the structure of unalloyed zirconia is monoclinic, whereas it assumes a tetragonal form at temperatures between 1170 and 2370°C and a cubic structure above 2370°C and up to the

melting point. Alloying pure zirconia with stabilizing oxides, such as CaO, MgO, Y_2O_3 or CeO_2, allows the retention of the metastable tetragonal structure at room temperature.[83] Dental procedures, such as grinding or sandblasting, can trigger a tetragonal to monoclinic transformation in the surface region.[84] This transformation is accompanied by a substantial increase in volume (~4.5%) that induces surface compressive stresses, thereby closing any crack tips and enhancing resistance to further propagation. This characteristic, known as transformation toughening, increases the fracture strength and fracture toughness of Y-TZP ceramics compared to other dental ceramics.[85] On the contrary, increased phase transformation toughening may alter the phase integrity of the material and increases the susceptibility of the material to low-temperature degradation (LTD), which is also known as ageing. Therefore, a narrow range exists between improvement and destruction of mechanical properties.

The ageing process depends on several microstructure features, such as porosity, residual stresses, grain size and the stabilizer content of the processed material. A decrease in grain size and an increase in stabilizer content retard the transformation process.[86] According to the literature, the critical grain size ranges from 0.2 to 1 μm depending on the Y_2O_3 content; a grain size larger than 1 μm exhibits a large amount of tetragonal-monoclinic transformation, whereas with a grain size below 0.2 um, no tetragonal-monoclinic transformation can occur, resulting in reduction of fracture toughness. LTD results in an adverse cascade reaction involving Y-TZP grain pull out, roughening of the surface, increased wear and microcracking. When the microcracked and damaged zone reaches the critical size for slow crack growth to proceed, degradation

in mechanical properties of the material occurs. However LTD of Y-TZP can be minimized by the addition of small amounts of silica, the use of yttria-coated rather than co-precipitated powder, the reduction of the grain size, increase in the stabilizer content or even the formation of composites with aluminium oxide (Al_2O_3).[87] However, the need for more advanced studies on the correlation between microstructure and LTD are still warranted.

Zirconia Implant Failure

Based on research in the field of Biomaterial Sciences, failures in Zirconia can be either chemical and/or mechanical where mechanical failure can occur either during the surgical placement of the implant, or subsequent functional loading. The manufacturing imperfections or flaws created during ceramic implant fabrication and subsequent surface treatment may compromise their strength. Material flaws usually assume the form of pores or microcracks of a submillimetre scale. Such cracks when combined with high bending moments or biomechanical overload, can initiate crack propagation and result in early implant failure. Bending load exerts a bending moment on the fixture cross-section at the crestal bone level.[88] Peri-implant bone resorption increases the crown to implant ratio, resulting in an increase in bending moment-induced forces and, when combined with lateral occlusal loading, it can result in premature implant failure.[89]

Geometrical implant design can also be a contributing factor for ceramic implants failures. Given the brittle nature of ceramics, all areas of excessive stress concentration

should be avoided and this includes, but is not limited to, the configuration of the thread design. Sharp, deep and thin threads as well as sharp internal line angles represent areas of stress concentration that can enhance the likelihood of crack propagation and implant failure.[90] During surgical procedures, difficulties can be encountered when inserting the implants in dense hard-type bone. If hand torqueing is needed for final insertion of the implant and the applied forces are not purely rotational in nature, bending forces may be generated, resulting in implant failure. LTD can account for the failure of zirconia implants through a process similar to "subcritical" or "slow-crack" growth (SCG).[91]

A number of studies investigated the influence of surface microtopography on the osseointegration of zirconia implants. In a rabbit model, Oliviera et al. analyzed the bone tissue response (histologically and biomechanically) to Y-TZP implants with different surface topographies, using oxidized titanium implants as controls. The removal torque values were significantly higher for surface-modified zirconia and titanium implants compared to machined-surface implants, with no significant difference regarding bone-to-implant contact between the two different materials.[92] Gahlert et al. compared the peri-implant bone formation and mechanical stability of surface-modified zirconia implants with sandblasted and acid-etched titanium implants. Similar degrees of bone implant contact and bone volume density was found for all of the implants, despite the fact that the titanium surface was significantly rougher than the tested zirconia surfaces.[93] However, titanium implants were found to have a higher removal torque resistance, probably due to the difference in the surface roughness.[94]

Nanostructured Surfaces

Nanostructured surfaces ranges from 1 to 100 μm and these surfaces may cause early integration of tissue surface with the environment. In an *in vitro* study, Mendonca et al. reported that nanostructured surfaces supported a significantly large extension of the fibrin clot formation[95] and in another study, the BIC was found to range between 55% to 96%, six to eight weeks after implantation. Peroxidation (using H_2O_2) or acid oxidation, (such as with hydrofluoric acid) have also been used to create nanotopography. The use of peroxide with acid etching has been shown to create novel nanostructures of amorphous titanium oxide on the implant surface. The treatment of the implant surface with H_2O_2/HCl increased the adsorption of RGD (Arginylglycylaspartic acid) peptides onto the surface followed by passivated surfaces (30% HNO_3) and heat-treated surfaces. These surface treatments increased the mineralization in the same order[96] and treatment with hydrofluoric acid also created discrete nanostructures on TiO_2 grit blasted surfaces.[97] Several cell culture studies, preclinical investigations and clinical studies support the observation that hydrofluoric acid treatment of TiO_2 grit blasted titanium implants is associated with rapid bone accrual at the implant surface. However, complex chemical changes induced by these methods may require careful inspection.[98]

Alkali treatment with NaOH produces a sodium titanate gel layer on the Ti surface, which was also seen with other materials such as zirconium and aluminum. Titanium oxide nanotubes chemically treated with NaOH, accelerated HA crystal growth

in a simulated body fluid and the kinetics of HA formation was significantly accelerated by the presence of the nanostructure associated to the NaOH treatment.[99]

The deposition of nanoparticles onto the titanium surface represents a fourth approach to imparting nano-features to a titanium dental implant. Sol–gel transformation techniques achieve deposition of nanometer-scale calcium phosphate accretions to the implant surface. Alumina, titania, zirconia and other materials can also be applied.[100]

Lithography and contact printing techniques can also be used and several different shapes and materials can be applied over the surface. However, these approaches are labor intensive and require considerable development prior to clinical translation and application on implant surfaces.[101] One of the main concerns related to coating the implant surface is the risk of coating detachment and toxicity of related debris.[102] Wei et al. compared the relationship of particle size, cell viability and proliferation of nanoparticles to micron-particles and found that nanoparticles of titania and alumina had less negative impact on cell viability and proliferation.[103]

There may be an advantage to nanoscale modification of surfaces, using sol–gel coating methods. The quantum interaction of high electron density at the atomic level can enforce high bond strength between the substrate and nanoscale coating.[104] Nanosurface changes alter the implant surface interaction with ions, biomolecules and cells, which can favorably influence molecular and cellular activities and alter the process of osseointegration.[105] Cell culture studies reveal that nanoscale topography promotes the

osteoinductive molecular program for adherent osteoprogenitor cells. Additionally, nanoscale alterations may promote bone-bonding behavior at the titanium–bone interface, and nanoscale modification of titanium endosseous implant surfaces enhances interfacial bone formation measured as bone-to-implant contact.[106]

Novel Surfaces (Biodenta Surface Treated Implants)

The integration of dental implants with the alveolar bone relies not only on mechanical force, but also on the biological surface bonding force, thus achieving integration. To support osseointegration of a new dental implant, Biodenta implants, a proprietory anodization process occurs during the dental implant manufacturing. This Biodenta Surface Treated (BST) implant consists of an amorphous titanium oxide layer that has surface roughness enhanced through open porosity. The osteoconduction process allows bone growth onto the surface. The Biodenta implant was designed with features that make it a good implant choice for many clinical applications, as the design allows for high levels of initial stability and a reliable prosthetic platform for most restorative situations. Enhanced osseointegration is achieved by surface activation through the anodization process and porous structure is achieved via the osteoconduction process.

As mentioned earlier, significant surface roughness is important in providing effective surface for bone implant contact, cell proliferation and good mechanical properties. Numerous studies were performed to modify titanium surface in order to enhance osseointegration. The implant modifications can either be achieved by additive

or subtractive methods. The important methodologies that have been utilized in the surface treatment of dental implants and their characteristics are summarized in Table 1.

Table 1. Surface characteristics, advantages and disadvantages of various commercially available implant surfaces

Surface	**Characteristics**	**Advantages**	**Disadvantages**
Nanotite	• Lowest surface roughness; relatively smooth • Roughness same for flank, top and valley • High Ti alloy content • Dual acid etched surface • Discrete nanocrystals of calcium phosphate adsorbed on implant surface	Long term documented success and survival rates De novo bone formation Better early osseointegration Success rate 95.3%- 99%	Most of the studies are preclinical and in vitro studies with no randomized clinical trial. Screw loosening and screw fracture in short implants

Table 1. Surface characteristics, advantages and disadvantages of various commercially available implant surfaces (Continued)

Tiunite	• Surface roughness is highest at flank than valley and lowest at top • Regularly organized figure 8 microstructure • Moderately rough surface with multiple cracks in the surface layer • Lowest Ti content • This surface is created by anodization (electrochemical process carried out in presence of an electrolyte)	Long term documented success and survival rates High BIC (93.3%- 94.7%) High success rate (97.1%-99.1%) Enhanced osseointegration by increasing platelet activation and red blood cell agglomeration	No ultrastructural studies of the interface between the bone and the implant surface has been found

Table 1. Surface characteristics, advantages and disadvantages of various commercially available implant surfaces (Continued)

Osseospeed	• Surface roughness is same for flank, valley and top • High surface roughness • Two-phase microstructure • Acid etched surface • Grit blasting with TiO_2 • Fluoride is incorporated in oxide layer with hydrofluoric acid etching • High amount of Titanium, containing elevated levels of oxygen	Early bone healing Fast bone healing process De novo bone formation Long term marginal bone maintenance 96%- 97.3% success rate	Short term follow up studies

Table 1. Surface characteristics, advantages and disadvantages of various commercially available implant surfaces (Continued)

SLActive	• Highest surface roughness • Honeycomb type microstructure • Grit blasting with alumina oxide and acid etched in presence of nitrogen • Surface roughness highest at flank than top and least at valley • Highest Ti content	High alkaline phosphatase activity in osteoblast like cells Increase in protein adsorption ad healing factors on surface High BIC Less bone resorption Success rate 97.5% - 99% (as per different studies)	A few long term follow up studies Most of the studies are preclinical and in vitro studies with very few randomized clinical trial No ultrastructural studies of the interface between the bone and the implant surface has been found

Table 1. Surface characteristics, advantages and disadvantages of various commercially available implant surfaces (Continued)

Osseotite	• Dual acid washed • The extent of acid washed surface is available in three choices • Osseoconductive • Osseotite 2 implants differ from osseotite implants in the apical geometry	Long term documented success rates Increased contact osteogenesis Enhancement of bone integration	No ultrastructural studies of the interface between the bone and the implant surface has been found

Peri-Implant Mucositis versus Peri-Implantitis

The increasing use of dental implants has led to a rise in complications due to peri-implant inflammation involving the soft tissue and bone, which ultimately leads to the loss of the implant. *In vitro* studies have shown that osteoblastic cells attach, spread and proliferate more rapidly on smooth surfaces than on rough ones.[107] However,

osteoblasts present higher rate of differentiation and matrix mineralization and higher production of growth factors in the presence of rough substrates.[108] Additionally, bone matrix proteins, alkaline phosphatase and osteocalcin, important indicators of osteogenic differentiation and bone tissue formation have shown to be expressed at higher levels on rougher titanium surfaces.[109] Therefore, moderately roughened surfaces show increased osteoblast proliferation.[110,111,112] However, this added roughness provides an optimum habitat for initial periodontal pathogen adhesion, which may cause peri- implant disease.[113] Peri-implant disease can be categorized as two forms: peri-implant mucositis and peri-implantitis and roughened surfaces show increased incidence of both forms of the disease. Peri-implant mucositis is an inflammatory process affecting the mucosa and is characterized by redness, and bleeding upon probing when a pressure of <25 Newtons is applied with a periodontal probe. Peri-implantitis, on the other hand, is an inflammatory process that not only affects the soft tissue, but also the bone. This process is characterized by changes in the level of crestal bone, presence of suppuration, bleeding on probing, and possible deepening of peri-implant pockets.[114] Both disease processes are similar in that they are both inflammatory reactions in the tissue surrounding the implant. The contrast between the two forms lie in the fact that peri-implant mucositis is an inflammatory disease process that is confined within the soft tissues around the implant with no apparent bone loss during initial bone remodeling and bone healing.[115]

Smeets et al. reported that the prevalence of mucositis and peri-implantitis ranged from 5% to 63.4% due varying study designs that have different population sizes with varying risk and statistic profiles. However, due to differing study designs, it is difficult

to determine the exact consensus figure to prevalence of peri-implant diseases. Studies reporting incidence of the peri-implant diseases also face similar challenges as the prevalence studies.[116] Based on the Consensus Report of the Sixth European Workshop in Periodontology, the incidence of mucositis was reported to be up to 80% while peri-implantitis was found to be between 38% and 56%.[117] Therefore, additional studies with a universally accepted study designs are required to accurately determine the incidence, as well as prevalence of these conditions.

Microbial Etiology of Peri-Implant Disease

Microbial colonization is the major predisposing factor for the development of peri-implant diseases. Animal and human cross-sectional studies have shown that bacterial colonization of implants is similar to that on teeth. These observations indicate that peri-implant disease is an analogous and/or related process to periodontal disease development as periodontal pathogens may emerge from residual dentition.[118] Peri-implantitis is a poly-microbial anaerobic infection that involves a spectrum of pathogenic microorganisms associated with periodontitis, such as *Prevotella intermedia, Prevotella nigrescens, Streptococcus constellatus, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia.*[119] However, in contrast to periodontitis, peri-implantitis is also associated with opportunistic microorganisms (e.g. *Staphylococcus aureus, Enterobacteriaceae*. *Candida albicans, Pseudomonas aeruginosa*) that are not found in the periodontal disease microbiota.[120,121] *S. aureus* specifically plays the primary role in the development of peri-implantitis.[122] In

fact, DNA-DNA hybridization studies have shown that *S. aureus* DNA counts are greater on dental implants than on natural teeth.[123] The presence of *S. aureus* in peri-implantitis was confirmed by Leonhardt et al. using culture dependent methodsLeonhardt et al. using culture dependent methods confirmed the presence of S. aureus in peri-implantitis.[124]

Similar to periodontitis, the first step in the development of peri-implantitis involves adherence of plaque microorganisms to the implant surface. This process stimulates gingival epithelial cells to release chemotactic peptides that attract polymorphonuclear neutrophils to the peri-implant pockets. The microbial damage to epithelial cells triggers release of inflammatory cytokines into the peri-implant crevicular fluid. The release of cytokines attracts more neutrophils, and other leukocytes to the affected sites where they degranulate releasing enzymes that cause tissue damage and gingival inflammation. The longer the duration of the inflammation, the more likely the inflammation will expand to the marginal gingiva and ultimately cause bone loss, which is the primary feature that differentiates peri-implantitis from peri-implant mucositis. Additionally, stromal cells may also be involved in the pathogenesis of peri-implantitis by upregulating vascularity and matrix breakdown, which in turn promotes the infiltrates to migrate to the inflamed sites where they are maintained over the course of the process.[125]

Risk Factors for Peri-Implant Disease

Numerous risk factors are associated with the development of peri-implant diseases, most of which are comparable to those for periodontal diseases. Among those identified by Smeets et al. are smoking, (with higher risk of complications in combination with positive IL-1 genotype polymorphism); history of periodontitis; lack of oral hygiene and maintenance; systemic diseases; iatrogenic causes; soft tissue defects; and a history of implant failure. While some of the aforementioned factors are still in contention, other risk factors are established as important contributors to peri-implant disease.[126]

Smoking is the most cited risk factor for peri-implant disease. A recent systematic review and meta-analysis by Sgolastra et al. demonstrated a significantly higher risk of peri-implantitis in smokers compared to nonsmokers.[127] Smoking is associated with an increase in the expression of the receptor of advanced glycation end products (RAGE) in gingival tissues. In the body, nicotine metabolizes to a metabolite called nor nicotine that upregulates RAGE expression in the gingiva of smokers. The upregulation of RAGE leads to the secretion of cytokines and reactive oxygen species that eventually result in alveolar bone loss.[128] Moreover, smoking and presence of IL-1 gene polymorphism were shown to have a synergistic effect that results in an increased risk of peri-implantitis.[129] While a significant correlation between IL-1 gene polymorphism and peri-implantitis has been suggested by some studies, numerous others found no apparent associations between these conditions. Therefore, further in depth investigations are necessary to

determine if a person's genetics can be considered a potential risk factor for peri-implantitis.

History of periodontitis is the second most common risk factor for peri-implant disease and it has been shown that patients with a previous history of periodontitis had a significantly higher rate of peri-implantitis compared to patients without a history of periodontal disease.[130] However, findings from a study by Meyle et al. suggest that osseointegrated implants in nonsmoking patients with chronic periodontal disease placed on a steady oral hygiene maintenance regimen, displayed implant survival rates of up to 100% over a period of 10 years.[131] Further, since periodontal pathogens in peri-implantitis also play a part in the etiology of periodontitis, it is difficult to dispel the argument that peri-implantitis is more common in patients with a history of periodontitis.[132] While there is reasonable data suggesting the association between history of periodontitis and peri-implantitis, more studies should be conducted in the future to further strengthen the notion that these factors are linked.

While few systemic diseases have been suggested as potential risk factors, diabetes mellitus is a widely established risk factor for peri-implant disease. In patients with poorly controlled diabetes mellitus, the periodontal inflammatory conditions are worse than those in healthy patients with periodontal disease. The mechanism behind this process is due to the fact that chronic hyperglycemia interferes with the tissue repair process and host defense mechanisms. Furthermore, the end products from advanced glycation that accumulates in the periodontal tissues increase cellular oxidative stress and

proinflammatory cytokines production in serum, saliva, and gingival crevicular fluid. The increase in proinflammatory cytokines may jeopardize the osseointegration and long-term survival of implants in patients with chronic hyperglycemia. Therefore, maintaining glycemic control in diabetic patients is paramount when performing implant surgery for osseointegration and implant survivability.[133]

The use of cement-retained restorations is prevalent in implant dentistry as a more esthetic option and for enhancement of occlusal contacts. Therefore, it is important to consider that the inadequate removal of excess cement at the time of cementation of the restoration may become an iatrogenic risk factor leading to cement-induced peri-implantitis.[134] Linkevicius et al. demonstrated that when margins of restorations are placed 1.5-3 mm below the gingiva, the probability of the remaining cement in the peri-implant sulcus is high.[135] The consequences of residual cement in the peri-implant sulcus has been shown to be important, as the residual cement has been associated with peri-implant tissue inflammation, BOP, suppuration, radiographic bone loss, and patient discomfort.[136] As a result, shallow intracrevicular placements of the gingival margins are recommended to reduce the occurrence of peri-implant inflammation and related complications.[137]

Purpose of the Study:

The purpose of this study was to investigate how bacterial accumulation is affected by surface roughness. To that end, bacterial adherence on different implant surfaces with various roughnesses was evaluated.

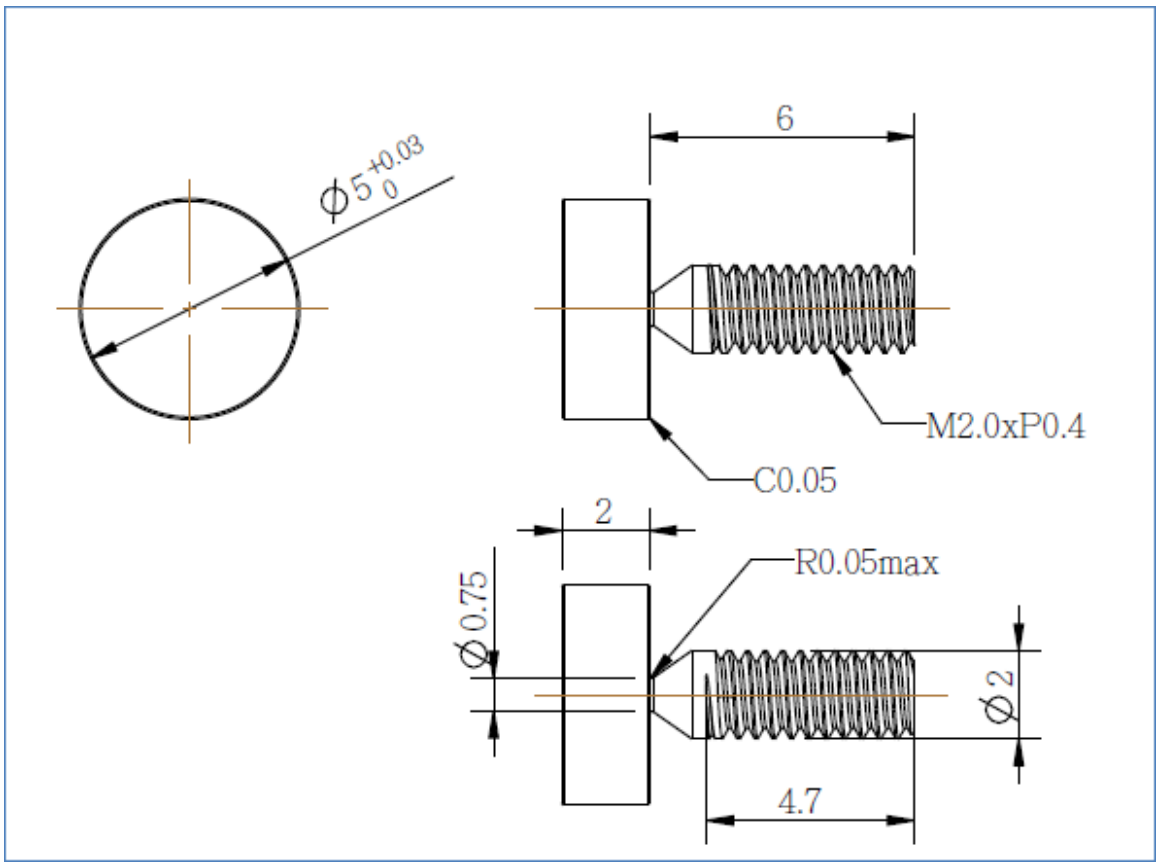
The different types of implant surfaces, listed by order of surface roughness, are:

1. Anodized yellow surface 0.3-0.5 µm
2. BST surface 1µm
3. Anodized yellow surface 0.8-1 µm
4. SLA surface 1.6 µm
5. Machined surface 0.3-0.4 µm

Miniature specimens of the 5 types of implant surfaces were fabricated by Biodenta Inc. Switzerland with the following dimensions:

The screw head is 5 mm in diameter and 2 mm in height ; shank is 1.3 mm in length with a diameter of 0.75 mm at its connection to the screw head, which then tapers to 2 mm diameter at its connection to the thread ; thread length is 4.7 mm with a major diameter of 2.0 mm and a pitch of 0.04 mm. All specimens were sterilized with Gamma rays. The diagramatic representation of miniature implant specimen is shown in Figure 1.

Figure 1. Diagram depicting an implant specimen



Hypothesis:

Null Hypothesis: There are no significant differences in the level of bacterial accumulation between the five different implant surfaces.

1. There is no significant difference in level of bacterial formation by *S. aureus* and *S. mutans*, on the five different implant surfaces.

2. There are no significant correlations between surface roughness of implants and level of bacterial formation by either *S. aureus* or *S. mutans* on each of the five-implant surfaces.

Research Hypotheses:

1. SLA surfaces will exhibit significantly greater accumulation of *S. aureus* and *S. mutans* biofilm than the other surfaces followed by BST (1 µm) and anodized yellow (0.8- 1 µm) surfaces and anodized yellow (0.3- 0.5 µm) and machined surfaces (.03-.04 µm) will have the significantly lowest level of bacterial accumulation.

2. There is a positive correlation between the surface roughness and bacterial accumulation for both *S. aureus* and *S. mutans* in all five groups, when considered together, and on each of the five different implant surfaces, separately.

Methods and Materials:

Five groups of different types of implant surfaces were selected and 20 specimens from each group were tested for bacterial adhesion. These implant surfaces included; Anodized yellow surface 0.3-0.5 µm, BST surface 1 µm, Anodized yellow surface 0.8-1 µm, SLA surface 1.6 µm and Machined surface 0.3-0.4 µm (Table 2).

Table 2. Surface materials of specimens

Materials	**Surface Roughness**
Anodized yellow surface	0.3- 0.5 µm
BST surface	1 µm
Anodized yellowsurface	0.8- 1 µm
SLA surface	1.6 µm
Machined surface	0.3- 0.4 µm

Strains and Growth Conditions:

The wild type *S. mutans* (UA159), and *S. aureus* (USA300) strains were stored at -80°C. *S. mutans* was maintained on Brain Heart Infusion (BHI) agar plates and grown in BHI broth (Teknova, Hollister, CA) anaerobically in an anaerobic jar with CO_2 gas pack at 37°C for 24 hours until early-stationary phase of growth. *S. aureus* was maintained on

Tryptic Soy Agar (TSA) and grown in Tryptic Soy Broth (TSB) at 37°C for 24 hours under aerobic conditions.

Bacterial Accumulation

Implant specimens were autoclaved prior to use. Bacterial cells from overnight cultures were harvested by centrifugation (8000 rpm, 18°C, 3 min) and cell pellets were washed three times with 10mM Dulbecco's Phosphate Buffered Saline (PBS) (Sigma Aldrich, St. Louis, MO) and resuspended in PBS. Optical density of cell suspensions was measured at 600nm using a spectrophotometer (BioMate 3S UV-Visible Spectrophotometer, Thermo Scientific, Waltham, MA).

Implant specimens from each of the 5 types were placed in eppendorf tubes, with one substrate per tube. Experiments were performed in duplicate for microscopic analysis of bacterial adhesion. Cell densities were adjusted to a final concentration of $1x10^8$ cells/mL in BHI for *S. mutans* and $1x10^8$ cells/mL in TSB for *S. aureus*. Bacterial cell suspensions in their respective media were added to each of the tubes with substrates and incubated for 48 hours in anaerobic jars (for *S. mutans*) and under aerobic conditions (for *S. aureus*). Cultures were incubated statically with no shaking.

Comparative Assessment of Bacterial Accumulation on Various Samples

Following incubation, substrates were removed and washed three times with PBS then placed in 50ml centrifuge tubes with 2mls PBS. Adherent bacteria were dissociated

from specimens via sonication and recovered cell suspensions were serially diluted in PBS. Aliquots (10 µl) from each suspension of both species were dotted in triplicate on respective agar media and plates were incubated for 48 hours at 37°C. Following incubation, colonies formed were counted and data was expressed as colony forming units (CFU/ml) as quantification of bacterial accumulation.

Scanning Electron Microscopy

For SEM, two implant specimens with adhering bacteria were rinsed twice with PBS then fixed in 2% paraformaldehyde, 2.5% glutaraldehyde in PBS, pH 7.4, for 1 hour at room temperature. Specimens were then washed in three changes of 0.1M PBS for a total of 30 min, post-fixed with 1% osmium tetroxide in PBS for 60 min and washed again in three changes of buffer. Dehydration of specimens was done using a series of graded ethyl alcohol, 30%, 50%, 70%, 90%, 100% for 10 min each and two more changes of 100% ethyl alcohol. Lastly, specimens were chemically dried by immersing sequentially in 2 parts 100% ethyl alcohol/1 part hexamethyldisilazane (HMDS) (Electron Microscopy Sciences, Fort Washington, PA) for 10 min, 1 part 100% ethyl alcohol/1 parts HDMS for 10 min, 1 part 100% ethyl alcohol/2 parts HDMS for 10 min then 2 changes for 10 min each with 100% HDMS. Specimens were air dried in a hood overnight, mounted on SEM pin mounts and sputter coated with 10 to 20 nm of platinum/Palladium in a sputter coater (EMS 150T ES). SEM images were taken in a scanning electron microscope Quanta 200 (FEI Co. Hillsboro, OR).

Statistical Analysis

A one-way analysis of variance (ANOVA) was used to test the first two hypotheses. Significant differences were further analyzed by Tukey's Honestly Significant Difference (HSD) test. Pearson's r was used to evaluate if a correlation exists between surface roughness and biofilm accumulation for data from all the groups together and each of the groups separately. A p value of ≤ 0.05 was considered significant.

Results:

Twenty samples from each of the five different implant surfaces of varying surface roughness were evaluated for differences in level of bacterial accumulation. Statistical analysis of results for all specimens demonstrated differences in the level of bacterial accumulation on the various surfaces. We first tested to see if there was a difference between implants surface roughness and accumulation by *S. mutans* or *S. aureus*. Secondly, a correlation was found between surface roughness and bacterial adhesion for *S. mutans* and *S. aureus.*

Differences between Surface Roughness and Bacterial Accumulation

Comparative ANOVA analysis of results between the five-implant surfaces for *S. mutans* adhesion, demonstrated a significant difference ($F=44.851$, $p= .0005$). Anodized surface

0.3-0.5 µm and machined surface 0.3-0.4 µm harbored significantly lower CFUs/ml than BST 1 µm, anodized yellow 0.8-1 µm and SLA 1.6 µm surfaces. CFUs/ml were significantly lower for SLA surface 1.6 µm than BST surface 1 µm. CFUs/ml of anodized yellow surface 0.8-1 µm are comparable to BST and SLA surfaces (Figure 2). Results from the statistical analysis of *S. mutans* recovery from the five-implant samples are summarized in Table 3.

Table 3. Summary of the statistical analyses for *S. mutans* recovery from the five implant samples

Implant surface	N	Mean	S.D.	F	p
BST 1µm	20	5.44 a*	0.57	44.85	0.0005
Anodized .8- 1 µm	20	4.91 a b	1.03		
SLA 1.6 µm	20	4.53 b	0.52		
Machined .3- .4 µm	20	3.59 c	0.37		
Anodized .3- .5 µm	20	3.21 c	0.29		

*Groups with the same letter are not significantly different.

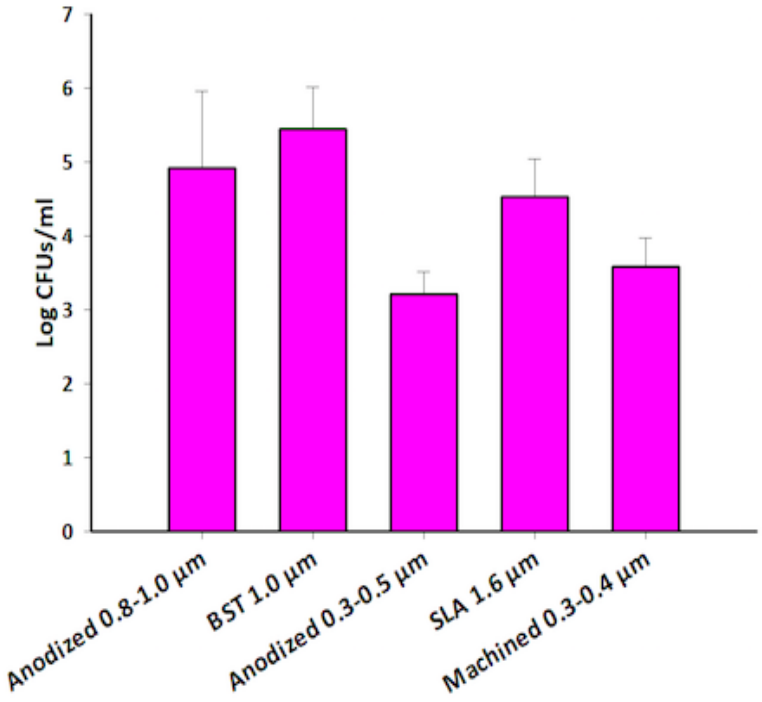


Figure 2: Comparison of *S. mutans* recovery from the various implant groups. Based on CFU quantification, *S. mutans* recovery from the various implants varied with BST 1 µm exhibiting the highest level of accumulation anodized 0.3-0.5 µm the lowest.

Similarly, for *S. aureus* ANOVA analysis demonstrated significant differences (F= 21.456, p=.0005) in levels of adhesion between samples. CFUs were significantly lower for machined 0.3- 0.4 µm and anodized 0.3- 0.5 µm surfaces than BST 1.0 µm, SLA 1.6 µm and anodized yellow 0.8- 1.0 µm surfaces. However, there were no significant differences in CFUs between BST, SLA and anodized 0.8- 1.0 µm surfaces (Figure 3).

Results of the statistical analyses of *S. aureus* recovery from the five implant samples are summarized in Table 4.

Table 4. Summary of the statistical analyses for *S. aureus* recovery from the five implant samples

Comparisons between mean log *S. aureus* CFUs from the five implant surfaces

Implant surface	N	Mean	S.D.	F	P
Anodized .8-1 µm	20	5.68 a*	0.36	21.45	0.0005
SLA 1.6 µm	20	5.56 a	0.38		
BST 1 µm	20	5.45 a	0.58		
Anodized .3-.5 µm	20	4.71 b	0.61		
Machined .3- .4 µm	20	4.46 b	0.62		

*Groups with the same letter are not significantly different

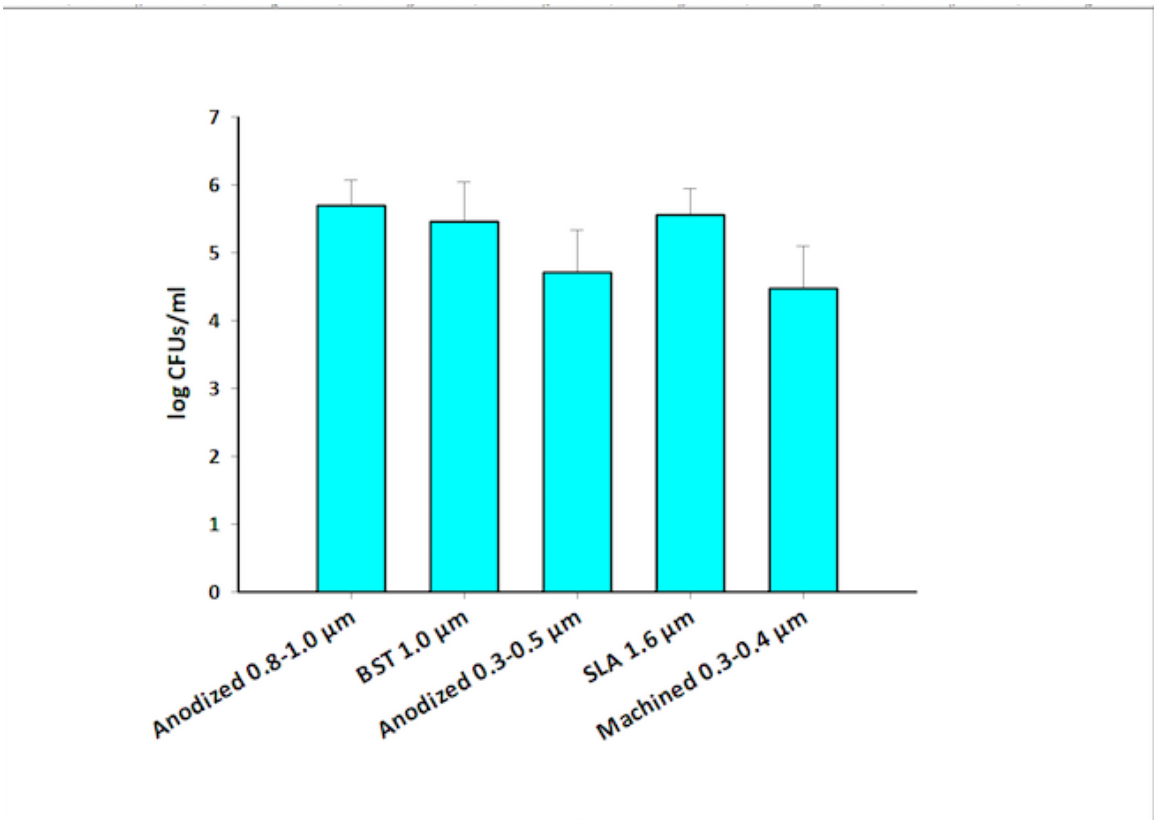


Figure 3: Comparison of *S. aureus* recovery from the various implant groups. Based on CFU quantification, *S. aureus* recovery from the various implants varied with anodized 0.8- 1.0 µm exhibiting the highest level of accumulation and machined 0.3- 0.4 µm the lowest.

Association between Surface Roughness and Bacterial Accumulation

Data was also analyzed for associations between the roughness of the surface of each specimen and level of *S. mutans* or *S. aureus* accumulation. Results demonstrated a significantly positive correlation. The correlation between surface roughness and CFUs

for *S. mutans* was significant r= .241, p= .008 (Table 5) The correlation between surface roughness and *S. aureus* was approaching significance r= .146, p= .073 (Table 6)

Table 5. Correlation between surface roughnesses and *S. mutans* recovery

	Surface roughnesses	**Mean log CFU SM**
Surface roughnesses		
Pearson Correlation	1	.241**
Sig. (1-tailed)		.008
N	100	100
Mean log CFU		
Pearson Correlation	2.41**	1
Sig. (1-tailed)	.008	
N	100	100

** Correlation is significant at the 0.01 level (1-tailed).

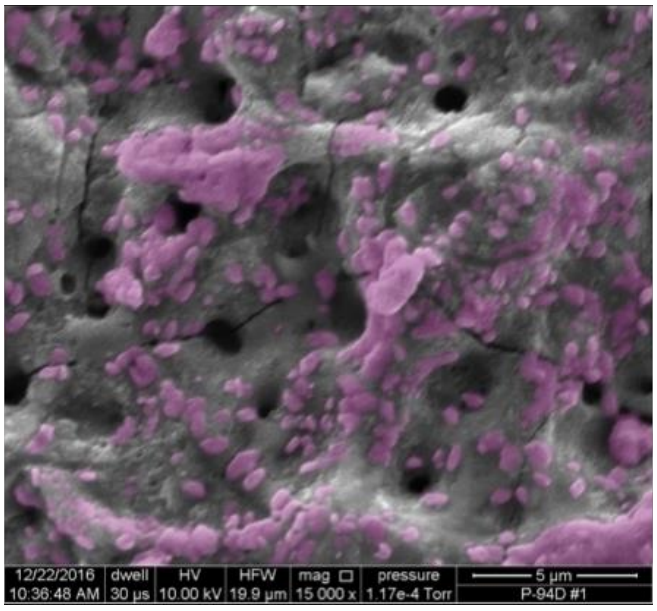
Table 6. Correlation between surface roughnesses and *S. aureus recovery*

	Surface roughnesses	**Mean log CFU SA**
Surface roughnesses		
Pearson Correlation	1	.146
Sig. (1-tailed)		.073
N	100	100
Mean log CFU		
Pearson Correlation	.146	1
Sig. (1-tailed)	.073	
N	100	100

Scanning Electron Microscopy

In order to visualize bacterial adherence on the surface of implants, representative samples from two of the five implant types (anodized yellow surface 0.8- 1.0 µm and machined surface 0.3- 0.5 µm), which demonstrated significant difference in *S. mutans* adherence were also subjected to SEM analysis. Consistent with microbial culture findings, images demonstrated higher affinity for *S. mutans* to the anodized surface 0.8-

1.0 µm (Figure 4) as compared to machined surface 0.3- 0.4 µm (Figure 5). Bacteria are colored pink in the figures for better identification.



***S. mutans* adherence to anodized yellow surface .8- 1um**

Figure 4. A false colored scanning electron micrograph demonstrating *S. mutans* (pink) adhesion on the surface of the anodized yellow 0.8- 1.0 µm material.

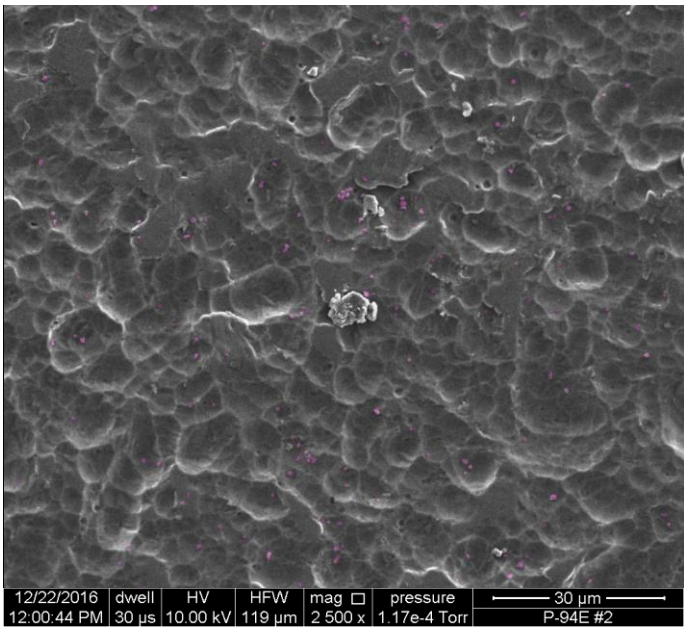


Figure 5. A false colored scanning electron micrograph demonstrating *S. mutans* (pink) adhesion on the surface of the machined surface 0.3-0.4 μm material.

Discussion

In this study, the effect of surface roughness (as quantified by Sa values) on bacterial accumulation (as quantified by CFUs/ml) was examined for five different implant surface groups. Based on the generated findings, the following null hypotheses were rejected:

- There is no significant difference in level of bacterial accumulation on the five different implant surfaces.
- There is no significant difference in *S. aureus* or *S. mutans* accumulation on the five different implant surfaces.

- There are no significant correlations between surface roughness of implants and *S. aureus* or *S. mutans* accumulation on each of the five-implant surfaces.

S. mutans Accumulation on the Five implant Surfaces

Comparative evaluation of *S. mutans* accumulation demonstrated significant variations between the various implant surfaces. As with surface roughness, although there were no significant differences between machined (0.3- 0.4 μm) and anodized (0.3-0.5 μm) surface groups, both groups exhibited significantly lower level of bacterial accumulation than SLA (1.6 μm), anodized (0.8- 1.0 μm) and BST (1.0 μm). SLA (1.6 μm) and anodized (0.8-1.0 μm) did not demonstrate significant differences but *S. mutans* accumulation on SLA (1.6 μm) was significantly lower than BST (1.0 μm) surface. Except for the SLA surface, the observed differences in bacterial accumulation appeared to be consistent with those for surface roughness values.

Numerous *in vitro* and *in vivo* studies have demonstrated that the surface texture has a significant impact on de novo plaque formation.[138,139, 140] Interestingly however, in our study, although SLA surface has maximum surface roughness, it did not harbor the

highest level of bacterial accumulation. It is widely reported in the literature that rough implant surfaces are beneficial for initial bone formation and generally enhance initial adhesion and subsequent colonization of oral bacteria.[141] However, interactive energy effects, e.g. substratum hydrophobicity, surface-free energy and charge, play a big role.[142] An *in vitro* study by Schmidlin et al. investigated early microbial adhesion and colonization of a multi species biofilm on seven differently processed titanium surfaces. Results from the study demonstrated that the modified SLA surface exhibited the highest trend for bacterial colonization and based on the findings, the authors concluded that surface roughness had a moderate influence on biofilm formation while wettability did not seem to influence biofilm formation.[143] These observations are in line with the findings from our study where surface roughness, although a considerable factor for bacterial accumulation, was not the most important factor. However, it is important to note that the study by Schmidlin et al. evaluated biofilm formation on titanium discs using 6 bacterial species (*Actinomyces oris* OMZ 745, *Veillonella dispar* ATCC 17748T (OMZ 493), *Fusobacterium nucleatum* KP-F2 (OMZ 596), *Streptococcus sobrinus* OMZ 176, *Streptococcus oralis* SK248 (OMZ 607), and *Candida albicans* OMZ 110), whereas in our study, only two bacterial species were included (*S. mutans* and *S.aureus*) which were evaluated separately and not in combination in a biofilm setting.

Consistent with what is described in the literature, at an international congress, the surface roughness of a dental implant was reported to be the primary factor influencing bacterial biofilm formation.[144] However, Hernandez et al. contradicted this conclusion in an in vitro study, where surface roughness was found to be less important in bacterial

adhesion than the physicochemical properties of the blasted particles, which modified titanium surfaces and affected the surface energy.[145] Thus, surface roughness is not the only factor influencing bacterial adhesion and plaque accumulation, but rather, the interaction of multiple factors of surface properties.

S. aureus Accumulation on the Five implant Surfaces

Comparative evaluation of accumulation of *S. aureus* demonstrated significant variations between the various implant surfaces. *S. aureus* accumulation on machined (0.3-0.5 μm) and anodized (0.3-0.4 μm) surfaces did not significantly vary however, both surfaces harbored significantly lower bacteria than BST (1.0 μm), SLA (1.6 μm) and anodized (0.8- 1.0 μm) surfaces. However, BST (1.0 μm), SLA (1.6 μm) and anodized (0.8- 1.0 μm) groups did not show any significant differences in *S. aureus* adhesion. In general, differences in bacterial accumulation appeared to follow the variations in surface roughness values. Thus, surface roughness may be an important factor in the promotion or inhibition of host cell and bacterial adhesion. *S. aureus* and *S. epidermidis* are a common cause of implant associated infections.[146] Peri-implantitis is associated with opportunistic microorganisms (e.g. *S. aureus, Enterobacteriaceae. Candida albicans, Pseudomonas aeruginosa*) that are not found in the periodontal disease microbiota.[147, 148, 149, 150, 151] While *S. aureus* may play the primary role in the development of peri-implantitis, it has been shown that all of these opportunistic pathogens have affinity for titanium and can cause infections such as osteomyelitis following implantation of orthopedic devices.[152] An *in vitro* study by Heitz-Mayfield et al.demonstrated the affinity

of *S. aureus* for titanium surfaces[153] and a number of clinical studies identified high levels of *S. aureus* at deep peri-implant pockets with the presence of suppuration and bleeding upon probing.[154] Although there is ample evidence suggesting the involvement of *S. aureus* in the initiation of some cases of peri-implantitis, further research into the role of this Gram positive facultative bacteria in the development of peri-implantitis is indicated.

Relationship between Surface Roughness and Bacterial Accumulation

Taken together, a direct correlation between surface roughness and bacterial accumulation was also demonstrated by our findings across all surface types. Significance was seen between surface roughness and *S. mutans* accumulation with a correlation coefficient r = 0.241, indicating a strong positive association, whereas the correlation between surface roughness and *S. aureus* adherence approached significance with a correlation coefficient r = 0.146. The values of correlation were low because average surface roughness was used rather than the actual values. Although it is important to determine correlation within the context of actual values, this goal is not within the scope of this current study. In contrast, the positive association found for all the groups is most likely due to the large range of values between the five groups and the higher sample size. It is important to iterate that while the results demonstrated a correlation, this does not necessarily indicate causation. Logically, it follows that macroscopically higher surface roughness would increase the propensity of a material to accumulate bacteria; however, this may also be affected by other surface characteristics

such as surface charge, surface energy and surface composition among others. Assuming that surface roughness is a critical factor in bacterial accumulation and has a causal relationship, lower surface roughness or relatively smoother surfaces may be expected to reduce bacterial attachment and accumulation. Conversely, where high-polished materials with reduced surface roughness limit initial bacterial adhesion, roughly textured implant substrata enhance plaque accumulation.

The five-implant groups with different surface roughnesses used in this study mimic the surfaces that are currently in clinical use. Since only one manufacturer fabricated the miniature implant specimens, the specimens did not have the exact same surface roughness; however, the levels of roughness fell within the average range. The machined surface served as control for this study, since this implant surface represents the starting point of implant surface design. The SLA surface represents Straumann's SLA surface with moderate surface roughness of 1.0- 2.0 μm. Three anodized surfaces of different surface roughness were examined to test the effect of different roughness due to anodization on bacterial colonization. The overall results indicated that these different surfaces exhibit differences in bacterial adherence where expectedly, bacteria tend to accumulate more on moderately rough surfaces than relatively smooth surfaces However, with moderately rough surfaces, roughness does not seem to be the only factor involved. Therefore, more in depth studies are warranted to further our understanding of the roles different implant surface properties play in promoting bacterial adhesion. Such insights are crucial as they may aid in the optimization of these physico-chemical characteristics with the goal of inhibition bacterial adhesion to implants.

Limitations

The main limitation of this study is that it is an *in vitro* study conducted under controlled conditions and therefore, it is not reflective of the complex environment in the oral cavity where various factors come into play such as pH, temperature, nutrients and importantly, host immune factors among others. Similarly, in our study bacteria were grown in defined microbial media, however salivary pellicles change the physicochemical property of the oral surfaces providing additional receptors for bacterial adhesion. Further, the various microbial species inhabiting the oral cavity exist in heterogeneous biofilms embedded within polysaccharides matrix, whereas our study included only two bacterial species that were studied separately. Another limitation is that a single manufacturer provided the implant samples used in this study and therefore are not accurately representative of other commercially available implants in terms of surface roughness, energy and charge in addition to wettability. These factors are important as they also impact bacterial adhesion to the surfaces.

Clinical Relevance

Dental implants largely vary in their surface properties, including structural and chemical compositions. To improve on the biological response to implants, modifications to surface topography such as surface roughness are continuously being made. Although surface roughness promotes bacterial adhesion, which may exacerbate peri implant-mucositis and peri-implantitis, host immune factors also play a key role in the disease

process as an active inflammatory immune response eventually leads to tissue damage. Particularly, exposure of an implant to the oral environment enhances disease progression due to increased bacterial adhesion and ensuing host-mediated tissue inflammation. Therefore, from a clinical standpoint, it is paramount that proper hygiene measures are meticulously implemented on daily basis to ensure the long-term health of the implant, regardless of surface properties. If these measures are not taken, accumulation of microbial biofilms on implants would be influenced by the titanium surface characteristics. In summary, bacterial adherence to moderately rough implant surfaces can be limited if meticulous oral hygiene and preventive care is maintained.

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