In Vitro Evaluation of the Effects of Multiple Oral Factors on Dental Implants Surfaces

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Presence of metal ions and debris resulting from corrosion processes of dental implants in vivo can elicit adverse tissue reactions, possibly leading to peri-implant bone loss and eventually implant failure. This study hypothesized that the synergistic effects of bacterial biofilm and micromotion can cause corrosion of dental implants and release of metal ions in vivo. The goal is to simulate the oral environment where an implant will be exposed to a combination of acidic electrochemical environment and mechanical forces. Four conditions were developed to understand the individual and synergistic effects of mechanical forces and bacterial biofilm on the surface of dental implants; In condition 1, it was found that torsional forces during surgical insertion did not generate wear particle debris or metal ions. In condition 2, fatigue tests were performed in a wet environment to evaluate the effect of cyclic occlusal forces. The mechanical forces applied on the implants were able to cause implant fracture as well as surface corrosion features such as discoloration, delamination, and fatigue cracks. Immersion testing (condition 3) showed that bacteria (*Streptococcus mutans*) were able to create an acidic condition that triggered surface damage such as discoloration, rusting, and pitting. A novel testing setup was developed to understand the conjoint effects of micromotion and bacterial biofilm (condition 4). Surface damage initiated by acidic condition due to bacteria (condition 3), can be accelerated in tandem with mechanical forces through fretting-crevice corrosion. Permanent damage to surface layers can affect osseointegration and deposition of metal ions in the surrounding tissues can trigger inflammation.

**Key Words:** dental implants, failure, fretting-crevice corrosion, modularity, osseointegration, oxide layer

**INTRODUCTION**

Despite the predictable success of commercially pure (cp) titanium (Ti) dental implants, 5%–11% of the implants still fail.1 This is a concerning issue, as the number of implants placed per year is increasing steadily to almost one million worldwide.2 The long-term clinical performance of implants is mainly dependent on the integrity of the surface titanium oxide (TiO2).3 This adherent, passive, TiO2 layer facilitates the process of osseointegration and also protects the bulk Ti from corrosion.4 The integrity and stability of the passive oxide layer can be affected by severe conditions such as acidic electrochemical environment, excessive stresses, and a combination of both factors.5 In particular, the oral environment possesses properties and functions that are important considering the possibility of corrosion process.

The oral environment has the potential to harbor over 600 different species of bacteria.6 Bacterial colonization on the surface of dental implants can occur immediately postimplant placement.7 The initial adhesion of planktonic bacteria can mature over time, leading to a polymeric network of multispecies biofilm.8 Bacterial biofilm has been observed in the peri-implant tissues of failed implants.9,10 Kumar et al11 noted an elevated level of the bacterial species *Streptococcus mutans* in implants with peri-implantitis as compared to the species associated with periodontitis. Previous studies have suggested that both microbial colonization and their metabolite products can damage passivity of the surface and disrupt osseointegration as well.12 Although attachment of bacteria and bacterial biofilm on implant surfaces is considered one of the most significant reasons for implant failure, only a few
studies have evaluated the role of bacteria in inducing damage to the TiO₂ layer. It is important to note that bacterial adhesion on the surface of dental implants can create an acidic electrochemical environment in two different ways: First, acidic metabolic products released by planktonic bacteria can reduce the pH and create an acidic condition. Streptococcus species, which have been reported to colonize dental implants, can release lactic acid as their metabolic byproduct. Chang et al. suggested that S mutans could cause corrosion in commercially pure titanium by using bacterial species suspended in a ringer solution as electrolyte in electrochemical tests. Second, a localized crevice region due to attachment of bacterial biofilm can lead to differential aerated zones on implant surfaces. In this localized region, there will be depletion of oxygen exposed to the surface decreasing the local pH. This unfavorable condition can lead to crevice corrosion and the release of metal ions in the oral environment.

Crevice corrosion can also take place in contacting interfaces such as connection regions of implants and implant-bone interfaces. Stagnation of fluid in these interfaces can deplete the ingress and egress of oxygen to the implant surface. In a retrieval study of titanium modular hip implants, it was observed that the pH in crevice areas was as low as 1. Another phenomenon of corrosion known as “fretting” can occur in these contacting interfaces due to micromotion, which can result in generation of metallic wear. It is known that cyclic occlusal loads imposed on dental implants can lead to micromotion against supporting bone and also micromotion of the modular parts of an implant. Dental implants typically have two modular parts: a modular connection between the implant and abutment, and a second modular area between the abutment and crown. These modular junctions have been widely investigated for their tendency to generate microgaps at the junction between the implant and the abutment, which are reported to range from 30–200 μm. Modular junction micromotion has been reported to cause microorganism leakage, crestal bone changes, mechanical instability, screw fracture and, in some cases, implant failure. However, the possibility of implant modularity and associated micromotion in triggering corrosion of dental implants has not yet been fully investigated.

The cyclic micromotion of modular parts can result in fretting corrosion and wear, which is retaliated with repassivation of the oxide layer under normal aeration conditions. However, in presence of a localized acidic environment created by bacterial biofilm, these processes may be accelerated, leading to fretting-crevice corrosion. Corrosion can lead to an accumulation of large amounts of metal ions in the oral environment, which may in turn trigger peri-implant inflammation and bone loss. Surface analysis of failed implants retrieved from peri-implantitis affected patients has previously been performed. Microscopic surface features revealed that surfaces experienced chemical as well as mechanical attack, leading to the permanent breakdown of the passive oxide layer. Wilson et al. observed the presence of titanium particles in soft tissue biopsies obtained from patients with peri-implant disease. Therefore, it is important to evaluate the individual and synergistic effects of bacterial colonization, and the mechanical forces that can lead to disruption of surface passivity and result in metal wear release in peri-implant tissues.

The purpose of this study was to simulate the oral environment where dental implants were exposed to a combination of electrochemical and mechanical factors. Four in vitro testing scenarios were developed to explore the effects of: (1) surgical insertion, (2) occlusal loading, (3) bacterial colonization, and (4) synergistic effects of occlusal loading and bacterial biofilm. In this study, the development of in vitro tests to evaluate the effect of bacteria, occlusal loads, and their synergistic effects will be described. Post-test surface analysis of implants obtained from mechanical fatigue test (condition 2) and bacterial immersion test (condition 3) will also be explained. Finally, a working prototype to study the synergistic effects of occlusal loading and bacterial biofilm (Condition 4) will be presented.

**Materials and Methods**

**Materials**

Poly (methyl methacrylate) based composite cement to secure the implant for mechanical testing. Bovine bone (Rudolph Market, Dallas, Texas) was used to insert the dental implants for load-to-failure test. Polyurethane foam blocks (referred to as “sawbones”) (Sawbone Inc, Vashon, Wash) of density 40 pounds per cubic foot (PCF) served as simulated bone model for making dental implant mount for mechanical testing. Phosphate buffered saline (PBS) packs (Sigma Aldrich, St. Louis, Mo) provided a simulated physiological medium for mechanical testing.

Streptococcus mutans UA 159 was chosen as the bacterial strain for immersion studies. Tryptic soy broth and solidifying agar (BD, Franklin Lakes, NJ) were used for agar cultures. Brain heart infusion (BHI) broth was utilized for broth cultures. GasPak EZ campy sachets and chamber with CO₂ indicators (BD) created the CO₂-rich micro-aerophilic growth conditions.

**Methods**

**Experimental Steps**

To understand the effect of each oral environment contributing factor on the surface oxide damage, four different experimental steps were developed, as detailed below:

- **Condition 1:** An insertion testing method for dental implants in simulated bone material (sawbones) was developed.
- **Condition 2:** A fatigue testing in simulated mild oral environment (0.01 M PBS) was developed.
- **Condition 3:** An immersion testing of dental implants in S mutans culture was performed.
- **Condition 4:** A fatigue test setup for dental implants immersed in bacterial culture was developed.

In this study, the development of in vitro testing methods is discussed for conditions 2–4, as condition 1 was investigated and reported in a recent study.
Implant Specimen Selection

Four sandblasted, large grit, acid-etched dental implants (referred to as I1-I4) (Straumann LLC, Andover, Mass), 4.1 × 10 mm were received for mechanical fatigue testing. In addition, a dental implant of the same dimensions and brand was used for quasi-static testing. One implant (I6) (4.1 × 10 mm) was utilized for the bacterial immersion test, and another—not subjected to immersion—served as a control (I5).

Condition 2: Mechanical Fatigue Test Simulating Mild Oral Environment

The main objective was to study the effects of occlusal forces on the implant surface under simulated mild environmental conditions using a mechanical fatigue test. “Mild environmental conditions” mean that no bacteria or acidic components were present in solution in the beginning of the experiment. The first step of the mechanical evaluation was to define upper and lower limits to simulate cyclic occlusal loading. A load-to-failure test was performed to define such limits.30

Load-to-failure testing. A dental implant was surgically inserted in bovine bone. A screw-retained crown, fabricated from a 51% gold alloy (UP1, Jensen Industries, North Haven, Conn) was then attached. Figure 1a shows the custom-made implant mount fixed to the base of the Materials Testing System (MTS, Bionix 370, Eden Prairie, Minn) end braces and loading point affixed to the top load cell. The test setup had the implant mount at a 30° inclination. Compressive axial load was increased at a rate of 0.05 mm per second until the implant fractured. Figure 1b shows post-test fractured implant.

The load-to-failure test generated axial force versus axial displacement graphs, as shown in Figure 2. Previous studies suggested that the upper limit of a fatigue loading cycle should be approximately 50% of the ultimate tensile strength. From this test, the ultimate tensile strength of the implant was found to be approximately 990 N. Hence, the upper limit of the fatigue test was chosen as 500 N, and 10% of the upper load limit was the lower limit for the test.30

Development of fatigue test in wet condition to simulate occlusal loading in mild oral environment. The fatigue test was designed per ISO standards.31 A dental implant was cemented inside a 40 PCF foam block with 3 mm exposure of the implant rough surface, as shown in Figure 3a.

Then, the implant was connected to a screw-retained crown tightened according to the recommended torque of 35 Ncm. A block spacer was cut with an inclination angle of 30° with respect to the horizontal plane to keep the implant mount tilted at the desired orientation (Figure 3a).

The dental implant mount and the spacer were clamped at the base of the MTS system and encased within a water bath chamber (MTS Envirobath 8.5 × 12 × 5.8 inches), as illustrated in Figure 3b. The other end made contact with a load cell. The water bath chamber was filled with 0.01 M PBS until the implant mount was completely immersed, as shown in Figure 3c. This provided a mild environment with normal pH of approximately 7. Cyclic compressive axial force was applied with load limits between 50 N and 500 N (as defined in the load to failure test) at 4 Hz for 2 million cycles. At the end of the fatigue test, the implants were retrieved and subjected to qualitative surface analysis using optical microscopy and scanning electron microscope (SEM) equipped with Energy Dispersive X-ray Spectroscopy (EDS).

Condition 3: Effect of Bacteria on the Surface Corrosion of Dental Implants

The test implant (I6) was immersed in a test tube containing 5 mL S mutans culture in BH broth for 60 days. This time period was chosen to perform a long-term immersion test that would allow for adhesion and formation of biofilm on the implant surface. During the immersion test, the pH of the test medium was monitored once every 2 days. Post-immersion, the surface of the implant was analyzed with different microscopy techniques. Surface of I6 was compared with a brand new untested dental implant (referred to as I5), which served as a control.

Condition 4: Synergistic Effects of Micromotion and Bacteria/Bacterial Biofilm on the Surface of Dental Implants

The fatigue test setup described in condition 2 was modified to design this synergistic test. In this condition, cyclic mechanical forces will be applied on implants immersed in bacterial broth cultures. A chamber was specifically designed for this test and was CAD/CAM fabricated using a 3D model printed out of acrylic resin material veroclear (Stratasys, Eden Prairie, Minn). Figure 4a and b show the sketch and picture of the dome-shaped chamber, respectively. The dimensions were accurate to perfectly fit at the base plate of the MTS inside the Envirobath chamber. The dome structure provides suitable environment for the bacteria to grow without contamination. Inlet and outlet ports on the sides of the chamber facilitated the flow of broth. The top views of the chamber (Figure 4c and d) present the provision at the top of the dome, which facilitates the entry of load cell to apply cyclic loading. A rectangular slot at the base platform of the dome structure will allow the placement of the implant mount. The implant mount was modified to perfectly snug-fit into that slot. Figure 4e shows a side view of sawbone with a ramp at the top, which was inclined at 30° to orient the implant for the fatigue testing.

Surface Analysis of Implants

Dental implants surfaces were analyzed postfatigue test with optical microscopy with magnifications in the range of 5–1000× (Keyence VHX-5000, Itasca, Ill) and a depth-up feature that captures 3D profiles of the specimen. Areas of interest analyzed with this technique were further inspected with SEM equipped with EDS.

RESULTS

The results for three different conditions developed for evaluating the implant surface are discussed.

Condition 2: mechanical fatigue test simulating mild oral environment

Mechanical Fatigue Test

Four implants were subjected to fatigue test. The desired performance requirement set for this experiment was 2 million...
cycles. However, the first implant (I1) failed at around 550,000 cycles. The implant prematurely fractured into 2 parts. The fracture was observed to occur along the contour of the 3-mm exposed surface (Figure 5a). The upper part of the implant consisted of the fractured 3-mm exposed rough surface, while the bottom part of the implant was actually cemented inside the sawbone block. The next three implants (I2–I4) (Figure 5b through d) survived the entire fatigue test of 2 million cycles with the revised load limits.

Microscopy Surface Analysis

Implants were imaged with optical microscopy before the fatigue testing. This served as a control for surface analysis. The smooth and rough surfaces exhibited no significant deformation when observed under high magnifications (Figure 6a). Post-testing, implants were imaged to understand the effect of occlusal forces on surface morphology. Characteristic surface features such as discoloration, fracture, surface delamination, and fatigue cracks were observed, suggesting surface damage. Figure 6b shows yellow/blue discoloration of the smooth surface of the implant (I1) (collar region). Similar discoloration feature was observed on the rough surface (Figure 6c) of all implants except I3. Further analysis of particular areas of interest performed using the SEM provided detailed features such as surface delamination (Figure 6d) and superficial fatigue cracks (Figure 6f). Delamination of the top layer was expected in I1 because of the occurrence of fatigue fracture. Surface delamination was also found in I2 in regions with evidence of discoloration. Black stains (Figure 6e) were observed in the SEM analysis in regions where the implants (I1, I2, and I4) displayed the characteristic yellow/blue discoloration.

The surface elemental composition of the implants (I1–I4) was analyzed with EDS. The elemental constituents of all implants are detailed in the Table. It is evident that the surface elemental composition of the implants mostly consisted of titanium (Ti), oxygen (O), and carbon (C). There were also trace amounts of other elements, such as vanadium (V), calcium (Ca), nitrogen (N), and phosphorus (P).

Condition 3: effect of bacteria on the surface corrosion of dental implants

The surface of the implant immersed in bacterial broth culture for 60 days (I6) was compared with a brand new dental implant (I5), which served as a control. Figure 7a shows the whole view of the control implant. Optical microscopy demonstrated a deformation free, intact surface (Figure 7b). The color-coded 3D map (Figure 7c) displayed a uniform distribution of color, which represented a pristine surface. Further analysis with SEM (Figure 7d) also showed a defect free and intact oxide layer. However, the implant immersed in bacteria (I6) showed visible color change in the full implant view (Figure 7e). In comparison to I5, I6 displayed change in color of the surface from grey to purple.
FIGURE 4. (a) A sketch explaining the dimensions of the custom made chamber. (b) Whole view of the 3D printed chamber. (c) Top view sketch displaying circular port of entry for mechanical forces and a rectangular slot for placing implant fixture. (d) Top view picture of the fabricated chamber. (e) Modified sawbone block used to make implant fixture that will snug-fit into the rectangular slot.

FIGURE 5. Low magnification images of implants post-fatigue test: (a) I1. (b) I2. (c) I3. (d) I4. Fracture observed with only I1.
FIGURE 6. Qualitative analysis of the surface post-fatigue test: (a) Control. (b) Discoloration at the implant-abutment interface of I1. (c) Yellow purple discoloration of the rough surface of I2. (d) Delamination of the top surface of I1. (e) Black taints at the rough surface I2. (f) Surface crack and propagation of fatigue crack in the smooth-rough interface of I3.

FIGURE 7. Control Implant (I5): (a) Whole view of the implant. (b) Flawless junction of smooth-rough surface of I5. (c) 3D depth up analysis showing a deformation free surface with uniform color distribution. (d) SEM image demonstrating an intact surface oxide layer. (e through h) Implant immersed in bacteria (I6). (e) Whole view of the implant with visible color change throughout the surface. (f) Surface color change from grey to yellow and blue. (g) Surface deformation in the form of micropits. (h) SEM image displaying surface deformation.
and yellow (Figure 7f). Figure 7g depicts the color-coded 3D image of the surface. The color distribution of I6 was not uniform as observed for I5. The color distribution changed in a series of raised regions (dark blue arrows) and depressions (black arrows) exhibiting micropit-like appearance. The diameter of these micropits varied from 15–25 μm, whereas the depth ranged from 10–35 μm. This severe surface deformation was prominent in the SEM image (Figure 7h) where the raised edges of the micropits are marked with red arrows.

EDS analysis of the implant immersed in bacterial culture detected the presence of titanium (Ti), oxygen (O), and carbon (C) with 63%–93% Ti, 9%–25% O, and 10%–18% C. The surface chemistry of the control implant showed the presence of Ti: 87%–92%, O: 3%–5%, C: 4%–6%, and nitrogen (N): 1%–2%.

**Condition 4: synergistic effects of micromotion and bacterial biofilm on the surface of dental implants**

In condition 2, the dental implant fixture was immersed in PBS. With that setup, the Environbath chamber of the MTS was able to house the volume of PBS required to completely immerse the implant. However, the fatigue setup needed to be improved to accommodate smaller volumes of bacterial culture, as the bacterial growth needed to be further controlled. Hence, a novel smaller chamber was designed that could be fit within the Environbath chamber of the MTS (Figure 8a). Figure 8b shows the inlet and outlet of the chamber, which enables flow of bacterial broth cultures. The constant flow rate of 20 rotations per minute will maintain the temperature of the microbial culture at 37°C. An opening centered at the top of this chamber (Figure 8c) was provided to facilitate the loading cell to enter the chamber setup and simultaneously apply cyclic forces on the dental implant mount. Figure 8c also shows a sawbone block (black arrow) immersed in the circulating deionized water in which dental implants will be placed for testing.

**DISCUSSION**

The purpose of this study was to develop different testing conditions to investigate the effect of mechanical and chemical
factors that could lead to surface damage of dental implants and release of metallic wear. Damage of the surface TiO₂ oxide layer can affect integration of soft and hard tissues with the implant. Corrosion and resulting dissolution of metal ions in the oral environment is considered to play a vital role in implant failure. Mouhui et al. has suggested that corrosion can be one of the underlying mechanisms of failure associated with dental implants. Henceforth, in this study, different testing conditions were developed to understand the individual and synergistic effects of mechanical and chemical attack on the surface. This region was exposed intentionally to simulate alveolar bone loss, which was in agreement with established surface. A torsional force was applied on the surface of the implant against simulated bone during the insertion procedure. Therefore, it was hypothesized that this mechanical force can lead to premature exfoliation of TiO₂. However, results of this study clearly concluded that the mechanical force involved in the insertion procedure did not lead to premature exfoliation of the surface oxide layer. To verify the effects of occlusal forces on the surface of the implant, a testing methodology was developed that enabled verification of multiple conditions in a single setup. Occlusal loads can lead to micromotion of a dental implant if osseointegration is not well established. Also, occlusal loads can lead to micromotion (fretting) of modular interfaces of the implant (abutment-implant and abutment-crown interfaces). Under conditions of normal aeration and physiological pH, the oxide layer of the metallic substrate is able to repassivate in the event of micromotion, protecting the surface against damage. However, in the presence of acidic environments, which can be created by oral bacteria or due to implant modularity, micromotion can lead to fretting-crevice corrosion. The hypothesis was proved with the surface analysis of the implants (I1–I4) post-fatigue testing. I1 did not survive the required fatigue performance of 2 million cycles, fracturing after 550,000 cycles. From Figure 5a, it can be inferred that the fracture of the top part was along the region of 3-mm exposure of the rough surface. This region was exposed intentionally to simulate alveolar bone loss, which was in agreement with established ISO standards for dental implant testing. It has been discussed that dental implants are less tolerable to nonaxial occlusal loading (loading experienced at 30° orientation) due to the absence of periodontal ligament. An important point to mention is the load limits selected for testing. After evaluation of the first results, it raised the possibility that the loads selected might have been too high and not representative of the loads achieved in the oral environment. However, studies published by Gibbs et al. and Koc et al. showed that mastication loads can vary from 150 N to 1280 N. The loads selected also took into account the worst case scenario (Bruxism) when loads are reported to be as high as 800 N. However, to understand the long-term performance of the implant without compromising on the average occlusal loading, the upper load limit was revised from 500 N to 450 N. The remaining implants (I2–I4) (Figure 5b through d) were able to complete the entire 2 million cycles designed for the fatigue test.

Post-fatigue testing, microscopy analysis showed corrosion features in the modular region of the implants, which supported the hypothesis. Figure 6b showed the characteristic yellow/blue discoloration. This characteristic discoloration represents the presence of titanium in different oxidation states such that blue indicates the presence of Ti⁵⁺, and yellow is linked to Ti²⁺. In this case, discoloration was only appreciated at the modular interface of the smooth surface of the implant (I1), where the implant comes in contact with the abutment. Literature reports on modular hip implants have already explained the ability of implant modularity to act as a crevice geometry, which can locally increase the acidity of the environment. Another area of evident discoloration of the rough surface (Figure 6c) was observed in all the implants except I3. In this case, discoloration was blanketed by a layer of cement, which was used in securing the implant fixture. This case illustrates that the crevice geometry between the implant-cement contacting interfaces, which produced a localized acidic environment that assisted in surface oxidation.

In both cases of discoloration, synergistic effects of micromotion and the possibility of acidic conditions due to localized crevice environment in the regions of the implants’ contacting interfaces could have played a major role. In this particular test involving a mild environment, the acidic condition was assumed to be induced by lack of oxygen in the crevice geometry created by the implant modular parts. More specifically, the junction between the abutment and implant is hypothesized to create the ideal conditions for triggering fretting-crevice corrosion. Another contributor factor could be the poor integration of the cement with the implant surface, which may have created microgaps that allowed micromotion to occur (Figure 6c). The threshold level for micromotion of an implant in the bone-implant interface has been reported in the literature to be approximately 150 μm, but fretting involves cyclic micromotion of amplitudes less than 100 μm. This clearly articulates that the role of fretting cannot be ignored with modular dental implants.

Furthermore, SEM/EDS analysis of implants post-fatigue testing corroborated the optical microscopy analysis. Regions of discoloration of the rough surface of the implants (I1, I2, I4) observed in the optical microscope were observed as black stains in the SEM analysis (Figure 6e). Suito et al. has suggested that these stains or dark phases on surfaces observed with SEM indicate breakdown of the oxide layer. As stated previously, catastrophic fracture was experienced during fatigue testing of I1. In addition, SEM analysis at higher magnifications displayed superficial fatigue cracks (Figure 6f) in both smooth and rough surfaces of I2 and I3. Surface fatigue cracks result due to long-term repeated loading, which in our case was the application of cyclic occlusal forces for 2 million cycles. These characteristic features are known to be associated with stress related corrosion, which can propagate and eventually result in surface damage. Similar superficial fatigue cracks were observed in our previous retrieval analysis of implants removed from patients due to peri-implantitis. On average, a person experiences 1 × 10⁶ chewing cycles per year, and this repeated loading over long term can lead to destruction of an implant surface. The EDS analysis of the rough surfaces showed high percentage of Ti (50%-85%) and low percentage of O (0%-10%). The complete EDS data analysis obtained for all investigated implants is presented in the Table. This explains breakdown of oxide layer (O-rich) and exposure of
the bulk (Ti rich). Corrosion resistance of Ti implants is dependent on the ability to form and reform this passive oxide layer. Breakdown of the oxide layer will lead to metal ions dissolution and particle debris deposition in the biological environment.

The effect of bacteria on the surface of dental implants was investigated with an immersion test. It was hypothesized that early colonizing Streptococcus species can create an acidic environment by releasing lactic acid as their metabolic product leading to damage of the passive surface. This was evident from the fact that a pH of 5 was observed in our S mutans broth cultures. This low pH was able to trigger surface damage changing the color and morphology of the surface. After 60 days of immersion, a characteristic blue/yellow discoloration was observed, which was similar to the feature observed in the implants post-fatigue test. The discoloration feature was only confined to the modular region and areas where marginal cement was present in the implants subjected to fatigue testing. However, surface attack due to acidic environment due to bacterial colonization was more pronounced with the discoloration spread throughout the surface of the implant (Figure 7a). Depth analysis (Figure 7c) showed that surface morphology was affected with the formation of micropits. Further analysis of this area of interest with SEM (Figure 7d) clearly illustrated severe deformation of the surface. Such surface deformations were previously witnessed in a retrieval analysis of failed implants. Inflammation, infection, and surface contamination associated with bacterial colonization on the surface of dental implants are associated with both early- and late-stage failure of dental implants. From this study, it was shown that bacteria can create an acidic electrochemical condition that can affect the implant’s surface morphology and surface chemistry. It is well known that the surface morphology and chemistry play a major role in the process of successful osseointegration. The higher percentage of titanium (Ti) and lower percentage of oxygen (O) on the surface detected in EDS analysis further corroborated damage of the oxide layer.

The results from the mechanical test showed that occlusal forces applied on dental implants in a mild environment can lead to damage of the surface. Therefore, it is important to understand the synergistic effects of bacterial biofilm and mechanical forces that will simulate the scenario in the oral environment to which dental implants are exposed. Henceforth, Condition 4 was developed to enable the simulation of oral environment. Dissolution of metal ions can be quantified by collecting aliquots of testing medium for every 100,000 cycles of fatigue test. In addition, this setup can also provide information about the possible leakage of bacteria through the modular connection. The versatility of this methodology is such that other experimental conditions in the oral environments—for example, presence of lactic acid and excessive concentration of fluorides—can also be simulated with the setup. Most importantly, this methodology can be employed to evaluate dental implants of different surfaces and dimensions. A working prototype of this setup was shown in Figure 8a through c. Preliminary results with this setup (data not shown) demonstrated that the system can accommodate a circulating broth medium maintained at 37°C, and the implant fixture can be simultaneously exposed to cyclic mechanical forces.

Some of the limitations of this study are based on the ability to maintain a contamination-free medium for the bacterial immersion test (condition 3) and the synergistic test (condition 4). It should be mentioned that the results obtained in this study are predominantly qualitative surface characterization. Another limitation is lack of true oral comparative biofilm mixed flora that may result in a different physicochemical behavior, compared to single species. More quantitative surface analyses such as X-ray photoelectron spectroscopy and electrochemical corrosion tests are to be performed in the future. These tests will provide quantitative information about surface chemistry and surface potential. Also in this study, implants of particular surface treatment and dimension were investigated. Therefore, future research will involve implants of different surfaces and dimensions.

**CONCLUSION**

Surface analyses of implants postbacterial immersion and fatigue tests demonstrated damage of the passive oxide layer. Permanent damage to the oxide layer can lead to release of bulk metal ions. Dissolution of metal ions in the oral environment could play a fundamental role in triggering diseases like peri-implantitis. Further analysis of dental implants with the methodologies developed in this study will help articulate the role of metal ion dissolution in the process of implant failure.

**ABBREVIATIONS**

BHI: brain heart infusion
EDS: energy dispersive X-ray spectroscopy
MTS: Materials Testing System
PBS: phosphate buffered saline
PCF: pounds per cubic foot
SEM: scanning electron microscope

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