Development of methodologies to investigate the effect of bacterial biofilm and micro-motion on the corrosion of dental implants

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Statement of Purpose: Corrosion of titanium dental implants (DI) has been associated with implant failure and is considered as one of the triggering factors for peri-implantitis [1]. Although, titanium (Ti) is corrosion resistant, due to its ability to form and reform passive oxide layers, Ti is highly susceptible to conditions of varying pH [2-4]. The presence of oral bacteria or adhesion of bacterial biofilm on the surface of DI may acidify the environment creating the ideal conditions to trigger corrosion. The goal of this study is to define the role of bacterial biofilm and implant micromotion and their conjoint effects in producing corrosion and permanent breakdown of the Ti oxide layer on the surface of DI. Five different conditions were developed to evaluate the effect of insertion, bacterial biofilm, micromotion and their synergistic effects.

Methods: 25 Sand blasted acid etched surfaced (SLA) 4.1 x 10 mm (Straumann USA LLC) DI were used with 5 implants assigned to each testing condition. *Condition 1*: Analyzed Ti delamination during simulated implant insertion in sawbone of increasing densities (10, 20, 30, 40 & 50 Pounds per Cubic Feet (PCF)). Particle analysis was performed with microscopy (Keyence VHX-2000) and X-ray diffraction (Bruker D8 Advance Powder X-ray Diffractometer). *Condition 2:* Simulated insertion of DI in 40 PCF sawbone, serve as a positive control for conditions 4 & 5. *Condition 3:* Immersion of DI in bacterial medium to study the effect of acidic milieu on DI surface. Bacteria strains associated with peri-implantitis were selected for this condition (Table 1). The bacterial strain with the best growth rate was chosen for following testing conditions. *Condition 4:* Analyzed the isolated effect of micromotion on the surface of the specimens. DIs were inserted in 40 PCF blocks at an angle of 30^o and 3mm simulating exposure of a DI. The setup was clamped at bottom of Material Testing System (MTS, Bionix-370) equipped with a bath chamber containing physiological saline medium (0.01M PBS). DIs were subjected to cyclic load ranging from 50 N to 500 N at a frequency 4 Hz for 2 million cycles [5]. *Condition 5*: Analyzed the synergistic effects of bacteria and micromotion that can cause fretting-crevice corrosion on the surface. The testing design included a small chamber inside the enviro-bath with inlet and outlet of bacteria and broth to provide a simulated oral environment for DI, which was simultaneously subjected to cyclic loading. Evaluation of surface condition at each step was performed with Scanning Electron Microscope (SEM, JEOL 5600) and digital microscopy.

Results: Simulated implant insertion revealed the presence of metal particles left in the sawbone after removal. Microscopy indicated that particles (arrowed black spots) were seen concentrated in 10 & 20 PCF blocks (Fig. 1a-b), whereas they were seen scattered in 30 & 40 PCF blocks (Fig. 1c-d). Insertion of DI in 50 PCF sawbone was not possible due to high insertion force required. Differences in the surface features between DI immersed in bacteria & control Brain Heart Infused (BHI) medium for two days was observed. Striking discoloration was seen in implants immersed in bacteria (Fig. 1e&1g). Implant immersed in BHI medium remained pristine with scratches on smooth surfaces (Fig 1f&1h). Cyclic loading on DI generated number of cycles versus axial force curves, which monitored the effect of micromotion with load dampening. The inclusion of bacterial medium acidifies the implant environment assisting with the evaluation of fretting-crevice corrosion.

Bacteria	pH
Control (BHI)	7
A.actinomycetecomitans, E.coli	6
S. mutans, E.faecalis, S.uberis, S.salivarius	5
S.sanguinis, S.gordonii	5-4

Table 1: Bacteria cultured in 1:5 C Brain Heart Infused (BHI) broth incubated at 37^o C shows ability of bacteria to reduce pH of growth medium.

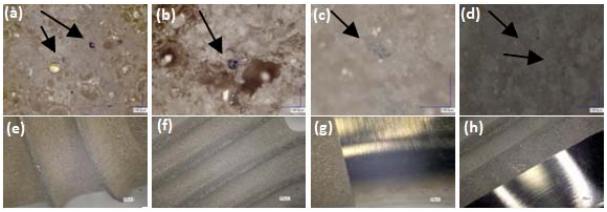

Figure 1[(a)-(d)] Particles in sawbone of increasing density: (a) 10 PCF (b) 20 PCF (c) 30 PCF (d) 40 PCF.[(e)-(h)] DI immersed in bacteria: (e) rough surface (g) smooth-rough junction; DI immersed in BHI control (f) rough surface (h) smooth-rough junction.

Conclusions: Table 1 indicates the ability of bacteria to acidify the implant surface and trigger oxidation. It was evident from the microscopic images that bacteria have an effect on the surface of the DI even after just 2 days. This indicates that bacteria alone can reduce the pH of the oral environment to the levels needed to initiate oxidation, as indicated by the discoloration indicative of Ti oxidation (Ti^{4+}). The insertion studies revealed the deposition of Ti particles in sawbones due to physical insertion and removal alone, which raises the possibility of Ti delamination in presence of cyclic loading. The results indicate that bacterial biofilms and cyclic loading may lead to accelerated dissolution and permanent breakdown of the oxide film. In vivo, released metal ions and debris may be associated with inflammation and bone loss around implants and the inability to obtain re-osseointegration.

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