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Biofilm and saliva affect the biomechanical behavior of dental implants

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ABSTRACT

Friction coefficient (FC) was quantified between titanium–titanium (Ti–Ti) and titanium–zirconia (Ti–Zr), materials commonly used as abutment and implants, in the presence of a multispecies biofilm (Bf) or salivary pellicle (Pel). Furthermore, FC was used as a parameter to evaluate the biomechanical behavior of a single implant-supported restoration. Interface between Ti–Ti and Ti–Zr without Pel or Bf was used as control (Ctrl). FC was recorded using tribometer and analyzed by two-way Anova and Tukey test ($p < 0.05$). Data were transposed to a finite element model of a dental implant-supported restoration. Models were obtained varying abutment material (Ti and Zr) and FCs recorded (Bf, Pel, and Ctrl). Maximum and shear stress were calculated for bone and equivalent von Misses for prosthetic components. Data were analyzed using two-way ANOVA ($p < 0.05$) and percentage of contribution for each condition (material and FC) was calculated. FC significant differences were observed between Ti–Ti and Ti–Zr for Ctrl and Bf groups, with lower values for Ti–Zr ($p < 0.05$). Within each material group, Ti–Ti differed between all treatments ($p < 0.05$) and for Ti–Zr, only Pel showed higher values compared with Ctrl and Bf ($p < 0.05$). FC contributed to 89.83% ($p < 0.05$) of the stress in the screw, decreasing the stress when the FC was lower. FC resulted in an increase of 59.78% of maximum stress in cortical bone ($p = 0.05$). It can be concluded that the shift of the FC due to the presence of Pel or Bf is able to jeopardize the biomechanical behavior of a single implant-supported restoration.

1. Introduction

Computer-based simulation such as the finite elements analysis (FEA) is used as a fast, predictive, and harmless tool to better understand the complexity of the mechanics involved in oral rehabilitation, especially in the screw–abutment–implant system and the maintenance of its stability (Caglar et al., 2011; Chun et al., 2006; Guda et al., 2008; Hanaoka et al., 2014; Khraisat, 2012; Lang et al., 2003). Most FEA studies consider the contact between the material surfaces as a key point to preserve the stability of the screw–abutment–implant system for long-term clinical success of dental implant rehabilitation (Gratton et al., 2001; Henry et al., 1996). Therefore, the friction and consequent wear between the materials could be influenced by parameters such as the roughness, Young's modulus, surface treatment, and the presence of a lubricant (Jorn et al., 2014; Souza et al., 2010a,b). The absence of these specific parameters, which are not generally evaluated in FEA studies, remains as a shortcoming of this methodology.

Although several factors may be associated with the instability of the screw–abutment–implant system, preload loss can be considered the most important one (Calderon et al., 2014; Jorn et al., 2014). Defined as the force responsible to keep the joint implant–abutment continuously tight during chewing, preload is generated after the placement of the screws. Factors such as the friction coefficient (FC) between the prosthetic contact surfaces (implant, abutment, and screw), may contribute to achieving and maintaining the adequate preload (Burguete et al., 1994; Gratton et al., 2001; Jorn et al., 2014) and system locking.

Therefore, the quantification of the friction between the prosthetic contact surfaces exposed to different situations present in the oral environment may increase the complexity of the simulations resulting in a more accurate FEA. In an attempt to achieve

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this, the role of the infiltrated saliva as a lubricant in the implant–abutment connection was the object of a previous study by Jorn et al. (2014). Although it was concluded that the saliva could harm the stress distribution of the dental implants, it should be pointed out that the friction coefficients they used were arbitrary, ranging from 0.2 to 0.5, considering that they did not determine the actual coefficient between the implant components.

Previous studies have already shown that bacterial colonization of the inner surfaces of implants can occur (Ricomini Filho et al., 2010; Koutouzis et al., 2014), and some studies have shown that such a colonization is able to reduce the friction coefficient when interposed between an alumina ceramic and titanium surface (Souza et al., 2010a,b). The resultant lower FC could contribute to increase the micromotion in the implant–abutment joint that could be transferred to prosthetic components and bone tissue (Gratton et al., 2001).

Therefore, the aim of this study was quantify the FC between materials used as abutments and implants in the presence of salivary pellicle (Pel) and biofilm (Bf) and evaluate the role of this FC in the biomechanical behavior of dental implants rehabilitation.

2. Material and methods

2.1. Experimental design

This study was approved by the Research and Ethics Committee of Piracicaba Dental School (register number 117/2013). Eighty-four titanium (Ti) discs surfaces were standardized and randomly allocated for trials according to the tribological couple. Titanium–titanium (Ti–Ti) and titanium–zirconia (Ti–Zr) ($n=14$) disc surfaces were divided into six groups according to the following conditions: titanium surface as control without Pel and Bf (Ti–Ti Ctrl and Ti–Zr Ctrl), Pel-coated discs (Ti–Ti Pel and Ti–Zr Pel), and multispecies Bf (Ti–Ti Bf and Ti–Zr Bf). The friction coefficient (FC) was analyzed using a tribometer with a Ti or Zr ball as counter body, followed by a scanning electron microscopy analysis. Additionally, the surface roughness of Ti and Zr surface discs ($n=4$) were characterized using an interferometer. After, virtual models were built to evaluate the biomechanical behavior of an implant-supported single crown restoration using FEA. Six models were obtained according to the abutments material (Ti and Zr) and the FC condition previously recorded (Ctrl, Pel, and Bf). The FC was simulated in the contact surfaces between implant–abutment, implant–screw, and screw–abutment from a Morse taper implant (4.1 × 11 mm) in the upper incisor region. A load of 49 N at 45° was applied, and the quantitative stress was calculated using the maximum stress and shear stress criteria for cortical and cancellous bone and equivalent von Misses criteria for implant, abutment, and screw.

2.2. Sample preparation

Eighty four titanium grade IV discs (12.5 × 2 mm) (Sandinox®; Sorocaba, SP, Brazil) were ground progressively with smoother aluminum oxide papers with 200, 320, 400, 600, and 1200 µm grid (Carbimet®; Buehler, Lake Bluff, IL, USA) in a horizontal polisher (model APL-4; Arotec®, Cotia, SP, Brazil). The number of discs was determined after a pilot study with a power of the test of 80%. The discs were ultrasonically cleaned with ethanol and purified water and finally dried for autoclave sterilization at 121 °C for 15 min (Souza et al., 2010a). The zirconia Y-TPZ discs were obtained commercially from CAD–CAM manufacturing (US Dental Depot®, Fort Lauderdale, Florida, USA). The disc preparation protocol was defined taking into account the surface roughness of commercial abutments during the pilot tests.

2.3. Surface roughness

The surface roughness of titanium and zirconia discs ($n=4$) was measured using a white light optical interferometer (New View 7300; Zygo Corp®. CT, USA). The following roughness parameters were obtained: average roughness (Sa) (µm); reduced peak height (Spk) (µm); reduced valley depth (Svk) (µm); and summit density (Sds) [represented by the number of peaks divided by the area (1/µm^2)].

2.4. Pel formation

Saliva was collected from healthy donors after obtaining written informed consent from each volunteer. For Pel formation, the Ti discs were positioned individually into a 24-well plate filled with 2 mL of a saliva solution (50 mL of non-stimulated and pasteurized saliva, 37.5 mL of deionized water, and 12.5 mL of saline solution). The Pel was allowed to form for 4 h at 37 °C by shaking (Guggenheim et al., 2001).

2.5. Bf development

A multispecies biofilm composed of the following strains was developed on the Pel coated-discs (Guggenheim et al., 2001; Shapiro et al., 2002): *Actinomyces naeslundii* OMZ745, *Streptococcus oralis* OMZ607, *Streptococcus mutans* OMZ918, *Veillonella dispar* OMZ493, *Fusobacterium nucleatum* OMZ598, and *Candida albicans* OMZ110. Briefly, each strain was spectrophotometrically adjusted to an optical density of 1.0 at 550 nm and a mixed-species inoculum was prepared with equal volumes of each strain. The biofilm was developed in a 24-well plate with modified fluid universal medium (mFUM) supplemented with glucose (0.15%) and sucrose (0.15%) (70% saliva and 30% mFUM). The plates were incubated anaerobically at 37 °C. The discs were washed in saline solution three times per day, and the medium was replaced at 16.5 h and 40.5 h. After 64.5 h, the discs were evaluated using the friction coefficient assays.

2.6. Friction coefficient assay

Ti discs of each group (Pel, Bf, and Ctrl; $n=14$) were fixed in a tribometer support (pin against disc) (Faculty of Mechanic Engineering: USP, São Carlos, SP, Brazil). The sliding test was performed with a vertical normal load of 10 N applied to each disc with a counter body of Ti or Zr ball (Y-TPZ), 5 mm in diameter. The test was carried out with a stroke length of 10 mm at 1 mm/s. These parameters were selected in a pilot study. The dynamic friction coefficient was evaluated with LabView® software (National Instruments®, São Paulo, SP, Brazil).

2.7. Scanning electron microscopy (SEM)

The discs covered with biofilm were fixed overnight in Karnovsky Fixative (2.5% glutaraldehyde, 2% formaldehyde, 0.1 M sodium phosphate buffer, pH 7.2), followed by dehydration in a series of ethanol washes (60%, 70%, 80%, and 90% for 5 min and 100% for 10 min) and allowed to dry under aseptic conditions. The discs were mounted on stubs, sputter-coated with gold and observed by SEM (JEOL JSM-5600LV; Peabody, MA, USA) at an accelerating voltage of 15 kV and angled in an inclination of 60° to evaluate the wear patterns.

2.8. Finite element assay

2.8.1. Model preparation

The anterior portion of an edentulous maxilla was reconstructed using medical tomography in the InVesalius® software (CTI®, Campinas, SP, Brazil). The STL images (stereolithography) were exported to the Solidworks software 2013® (Dassault Systèmes Solidworks®, Waltham, MA, USA) for bone model construction. The model was composed of cancellous bone surrounded by 1.5 mm of cortical bone corresponding to type III bone quality. Single crown rehabilitation was simulated in the anterior region of the maxilla and supported by a Morse taper implant (4.1 × 11 mm). The CAD model of an anatomic abutment (through-bolt) and a screw model were provided by the manufacturer (Neodent®, Curitiba, PR, Brazil). All anatomic references of the crown were based on microtomography images. The crown was considered cemented-retained by a 0.50 µm thick cement layer. After the implant placement 1 mm below the bone, the prosthetic crown of an upper middle incisor was constructed. The virtual models were exported to Ansys Workbench 14.0 software® (Swanson Analysis Inc®, Canonsburg, PA, USA) for the mathematical analysis.

2.8.2. Mathematical analysis

The respective Young's moduli and Poison ratios used was as follows: Cortical bone; 13.5 GPa, 0.23 (Cicciu et al., 2014); Trabecular bone, 1.36 GPa, 0.31 (Cruz et al., 2009); Titanium for implant, abutment and screw, 110 GPa 0.35 (Cruz et al., 2009); Zirconia (Y-TPZ) for the abutment; 205 GPa, 0.22 (Coelho et al., 2009). The prosthetic crown was considered to be lithium disilicate (96 GPa, 0.23) (Albakry et al., 2003) luted by a resin cement (18.3 GPa, 0.30) (Li et al., 2006). Convergence analysis with 5% tolerance was achieved using a tetrahedral mesh with an element size of 0.50 mm. All models were considered homogeneous, isotropic, and linear elastics, except the prosthetic component contact materials that were considered non-linear elastics. The mean frictions coefficients obtained in the *in vitro* study were simulated in the contact prosthetic surfaces: head of screw to abutment, screw thread to implants thread, and the Morse taper abutment to implant internal surface. Six tridimensional models were obtained according to the abutments material: Ti (titanium abutment); Zr (zirconia abutment), and the FC between the surfaces: Pel, Bf, and Ctrl.

The boundary condition was defined by fixing the mesial and distal external bones surfaces. A load of 49 N was applied with an inclination of 45° at the palatal surface of the prosthetic crown (Korioth et al., 1997; Hidaka et al., 1999). The maximum principal and shear stress were calculated for bone and an equivalent von Misses stress was obtained for the prosthetic components and implants.