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In vitro analysis of prosthetic abutment and angulable frictional implant interface adaptation: Mechanical and microbiological study

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ABSTRACT

The aim of this study was to evaluate, *in vitro*, the microbiological sealing at the implant and different angles frictional prosthetic abutment interface, submitted or not to mechanical cycling, as well as the deactivation force and evaluation of the implant-abutment interface by scanning electron microscopy. For this study, the sealing capacity of eighty sets of abutments/implants of each angle, with and without mechanical cycling, with internal conical connection (locking tapper) (4.3 mm × 9.0 mm) constituted in Titanium alloy (Ti6Al4V), and stainless steel angled prosthetic abutment was evaluated (18Cr14Ni2.5Mo) according to ASTM F138-13a (Arcsys, FGM, Joinville, Brazil), 6 mm high and 4.2 mm in diameter at the coronary portion, and 3.5 mm high transmucosal, in 4 different angles (0, 5, 10 and 20°). After *in vitro* tests, 100% biological sealing was observed at the implant / prosthetic abutment interface within cycled and non-cycled conditions, for the straight, 5, 10 and 20° inclination groups. There was no statistically significant difference in the removal force of the prosthetic abutments at different angles, under non-cycled conditions; however, under mechanical loading, the deactivation force was significantly higher for straight prosthetic abutments than with 10 and 20° of angulation. Surface analysis revealed good adaptation between implants and abutments, and the presence of wear areas, independently of mechanical loading. It is concluded that the analysis of implant and prosthetic abutment interface revealed good adaptation between the parts, for all analyzed samples.

1. Introduction

Despite proven success in terms of osseointegration with dental implants, complications related to the presence of microorganisms in the oral cavity can cause therapy (Ricomini Filho et al., 2010).

It is well documented that in almost all implant systems used there is a microgap at the prosthetic abutment and implant body interface (Jansen et al., 1997). Even in microgaps smaller than 10 μm, situations considered good marginal adaptation of abutments and implants (Nascimento et al., 2012; Silva-Neto et al., 2012), bacteria colonization and penetration can occur, which can result in local inflammation, alveolar bone resorption and, consequently, implant failure (Broggini et al., 2003). In view of this problem, several designs of implant systems that provide hermetic sealing of the implant and abutment interface have been developed (Nascimento et al., 2012).

In this sense, internal conical connections are the most efficient in dissipating forces generated in the prosthesis and also in maintaining the torque and stability of the prosthetic abutment. Although there is not complete elimination of the microgap, these connections are more effective in sealing the implant-abutment interface (Koutouzis et al., 2011; Alves et al., 2014; Alves et al., 2016), being related to the locking form of the prosthetic abutment in conical connections (Alves et al., 2014; Alves et al., 2016).

Screw-retained is the most used method, however, loosening and fracturing of the screw still present aspects of disadvantage. To minimize this problem, the frictional cone Morse connection was introduced as an alternative to screw-retained systems. Retention is carried out by friction between the male and female cones, with internal taper of 1–3° between the surfaces, generating high contact pressure within the conical region, and consequent interlock between surfaces. As a result,

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the surface oxide layers break, enabling the fusion of the rough portions, known as "cold welding". Despite intimate frictional juxtaposition of abutments in this type of connection, chewing and the vectors of occlusal forces can reduce stability of the system, favoring bacterial infiltration into internal spaces of the implant (Zipprich et al., 2007).

Ideally, dental implants should be aligned vertically to axial forces during chewing (Ferraz et al., 2019). However, there may be clinical situations, such as disadvantaged bone anatomies, where the prosthetic abutment must be angled to allow prosthetic rehabilitation, despite showing increased stress on supportive implants, adjacent bone and the prostheses they support (El-Sheikh et al., 2018), in addition to aesthetic disadvantages.

Bearing in mind that in some clinical scenarios there is need for abutments with different angles from those available by manufacturers, strategies for use of metal alloys that allow the personalization of the angle of the prosthetic component, have been an alternative. Recently, an implant system (Arcsys™, FGM) was developed that allows the prosthetic abutments to be angulated up to 20°, thanks to the use of stainless steel alloy (American Society for Testing and Materials, ASTM F 138), that with the use of a manual device, allows amplifying the manual force in a set of levers, overcoming the mechanical resistance of the abutments, thus printing a predetermined angulation.

Notwithstanding the originality of the system that configures clinical advantage in terms of customization of the angulation favoring prosthetic rehabilitation, there are doubts regarding the sealing promoted in the implant/prosthetic abutment interface, due to the angulation process and mechanical resistance during abutment removal force, when submitted to masticatory force vectors. Thus, this study evaluated *in vitro* the microbiological sealing at the implant/prosthetic abutment interface in different angles, submitted or not to mechanical cycling, as well as the deactivation force of the same. The null hypotheses evaluated were: 1) that different angles frictional prosthetic abutments and implant interface would not affect the microbiological sealing even under mechanical cycling; 2) that the deactivation force would be similar among the groups; 3) that the morphological alterations in the internal surfaces of frictional morse taper connections would be similar, independently of the prosthetic abutments angulation and mechanical cycling.

2. Methods

2.1. Study design and sample preparation

For this study, the microbiological sealing capacity of the conical internal connection implant (4.3 mm × 9.0 mm) made of Titanium alloy (Ti6Al4V) and an angled prosthetic abutment made of stainless steel (18Cr14Ni2.5Mo) were evaluated, according to ASTM standard F138-13a (Arcsys, FGM, Joinville, Brazil), 6 mm high and 4.2 mm in diameter at the coronary portion, and 3.5 mm high in transmucosal, in 4 different angles (0, 5, 10 and 20°).

Eighty sets of abutments/implants were used, with 40 sets being cycled and 40 not being cycled, with 10 sets of each angle, as described in previous *in vitro* studies with the same sample size (Alves et al., 2014; Alves et al., 2016; Peruzetto et al., 2016). In addition, for each model, 3 implants with their respective prosthetic abutments were used as a negative control and 3 sets without the prosthetic abutments, as a positive control of microbial contamination.

For activation, two blocks of polyurethane density 40 PCF (Sawbones, Palo Alto, USA, material manufactured according to ASTM F1839-08) were fixed in a metallic holding device (vise) and drilling was performed with a 3.4 mm diameter and 11 mm long drill, at the intersection line of both (Fig. 1). The implants were adapted in the niches, and the samples positioned again in a vise to avoid oblique loads during abutment frictional activation (Asmarz et al., 2021). The activations for all proposed angulations were performed through the impact produced by the hammer body in the direction of the long axis of the implant and according to the manufacturer, who recommends 3 activations. This device, parallelly positioned to the trajectory of the impact body of the instrument, standardized the strokes, avoiding variations in positioning, which would cause decreased frictional retention.

All samples were activated at the same time, by the same operator, even those that would not be subjected to mechanical cycling.

2.2. Mechanical cycling

For mechanical cycling, the specimens were maintained on prefabricated bases that allowed the fixation and assembly of the samples for cycling, according to the recommendations of ISO 14801:2012 (Fig. 1). Each base had a final cylindrical configuration, measuring 2.3 cm in diameter, with different angles for fixing the specimen, for straight prosthetic abutments (0°) and with an angle of 20° - base with 30° between the long axis of the implant and the ground, for prosthetic abutments with an angle of 5° - base with 15° between the long axis of the implant and the ground; and for prosthetic abutments with an angle of 10° - base with 20° between the long axis of the implant and the ground.

The samples were submitted to mechanical cycling using an Elquip® fatigue machine (São Paulo, SP, Brazil). 500,000 cycles were performed per sample, which is equivalent to approximately 6 months in functionality (Cibirka et al., 2001), receiving a load of 120 N at 2 Hz frequency (Richter, 1995; Stern 1995), at a final 30° angle in regard to the

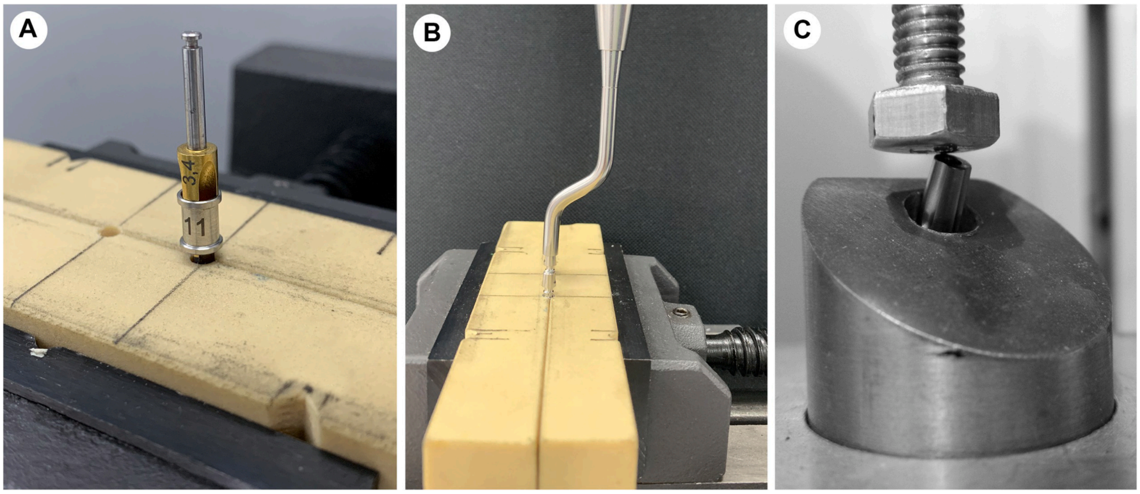

Fig. 1. Implant-abutment sets embedded in acrylic resin (A); activated by the hammer body (B); during mechanical cycling test (C).

long axis of the implant, for all studied groups.

2.3. Microbiological analysis and mechanical behavior

All samples were sterilized in ethylene oxide and immersed in tubes containing 75 ml of *Escherichia coli* suspension (American Type Culture Collection 25922), as previously reported in many *in vitro* studies (Alves et al., 2014; Alves et al., 2016; Peruzetto et al., 2016; Costa et al., 2017; Harlos et al., 2016). The bacterial suspension concentration was adjusted by optical density to 1.0 at 540 nm, which corresponded to a microbial concentration of 12×10^8 cells/ml. The suspension was incubated for 14 days (Quirynen et al., 2006; Waal et al., 2014), at 37 °C temperature under aerobic conditions, changing the culture medium every 48 h (Silva-Neto et al., 2012). After the incubation period, the implant/prosthetic component sets were removed from each vial and dried with sterile absorbent paper to remove excess bacterial broth. Each sample was rinsed, three times, using sterile distilled water and again dried with absorbent paper. The implant/abutment interface was disinfected with 0.25% peracetic acid by means of mechanical friction, 20 s for each sample, and subsequent drying with absorbent paper (Alves et al., 2016).

All samples were again fixed to their respective bases in order to separate the abutments from their respective implants, registering the force necessary to remove them. A traction machine with a 50 kg load cell was used (EMIC DL 2000, São José dos Pinhais, PR, Brazil), for separation at a speed of 1 mm/min, the values being recorded in Newtons (N).

In order to certify non-contamination of the external portion, before separation, each set was subjected to microbrush rub moistened in sterile 0.9% saline and immersed in Brain-Heart Infusion culture medium serving as control of external contamination (Alves et al., 2014; Harlos et al., 2016; Peruzetto et al., 2016).

Then, a thin moist microbrush was carefully rubbed over the most apical inner surface of each implant, to collect bacteria that could have penetrated the interface, and immersed in a tube containing 5 ml of sterile BHI broth and incubated at 37 °C for 48 h.

All procedures were performed by the same operator, previously trained through a pilot test, in a sterile environment.

2.4. Microstructural analysis of the implant/prosthetic abutment interface

Three samples of the implant/abutment sets, previously sterilized in an autoclave, were evaluated by scanning electron microscopy (microscope Quanta FEG 250, FEI, Germany), with 20 kV acceleration, and 500X magnification.

The implant and prosthetic abutment interfaces were analyzed at all evaluated angles, whether or not subjected to mechanical cycling, as well as the internal region of the implant after deactivation of the prosthetic component.

2.5. Statistical analysis

Initially, descriptive and exploratory data analyses were performed. This involved graphical analyses of the data and standardized residues, as well as application of the Shapiro-Wilk test to verify the normality of errors. Fisher's exact tests and two-way ANOVA were performed for microbiological and mechanical tests, respectively, using the R Core Team program package (Vienna, Austria), adopting a 5% significance level.

3. Results

3.1. Microbiological analysis

Table 1 shows the absolute and relative frequencies of media with turbidity, after 48 h, at different angles. No turbid medium was observed in any of the samples ($p = 1.000$), regardless of the angle or presence/absence of mechanical cycling.

Table 1
Absolute and relative frequencies of media with turbidity, after 48 h, at different prosthetic abutment angulations, in the presence or absence of mechanical cycling.

Angulation	Cycled	Not Cycled
Straight (0°)	0 (0%) Aa	0 (0%) Aa
5°	0 (0%) Aa	0 (0%) Aa
10°	0 (0%) Aa	0 (0%) Aa
20°	0 (0%) Aa	0 (0%) Aa

Different lowercase letters represent statistical differences at different angles, for each condition (cycled or non-cycled). Different uppercase letters represent statistical differences for each angle, comparing cycled and non-cycled.

3.2. Mechanical behavior

The removal force of the prosthetic abutments, at different angles, submitted or not to mechanical cycling are shown in Table 2.

There was no statistically significant difference in the removal force of the prosthetic abutments at different angles, under non-cycled conditions ($p > 0.05$). However, under mechanical loading, the deactivation force was significantly higher for straight prosthetic abutments than with 10 and 20° of angulation ($p < 0.05$). Additionally, a decrease in removal force was detected after mechanical loading at 20° prosthetic angulation than non-cycling condition ($p < 0.05$).

3.3. Microstructural analysis

The analysis of the implant and prosthetic abutment interface showed good adaptation between the parts, for all analyzed samples (Fig. 2).

The internal surfaces of the implants and the external of the prosthetic abutments revealed the presence of longitudinal grooves along the long axis of the implant, showing frictional activation between the parts, independently of mechanical loading (Fig. 3).

4. Discussion

In clinical situations where the implant was installed out of the ideal axial situation, the use of angled prosthetic abutments can present advantages in the rehabilitation of the patient. For this reason, the regulation itself provides analysis of the worst situation found in clinical use of implants, which explains the use of abutments with different models and lever arms, characterizing different situations, but used for the same purpose, installation of prosthesis on unitary implant. El-Sheikh et al.

Table 2
Mean and standard deviation for removal force (N) according to prosthetic abutment angulation, in the presence or absence of mechanical cycling.

Angulation	Cycled	Not Cycled	Mann Whitney test (uppercase letters)
Straight (0°)	249.26 (±22.20) Aa	272.26 (±19.70) Aa	p-value = 0.1495
5°	226.81 (±16.90) Aab	276.43 (±60.60) Aa	p-value = 0.2623
10°	197.45 (±16.00) Ab	267.36 (±77.50) Aa	p-value = 0.0547
20°	190.91 (±21.00) Bb	241.68 (±30.50) Aa	p-value = 0.0065
Kruskal Wallis (lowercase letters)	p-value = 0.0012	p-value = 0.4180	

Different lowercase letters represent statistical differences at different angles, for each condition (cycled or non-cycled). Different uppercase letters represent statistical differences for each angle, comparing cycled and non-cycled.

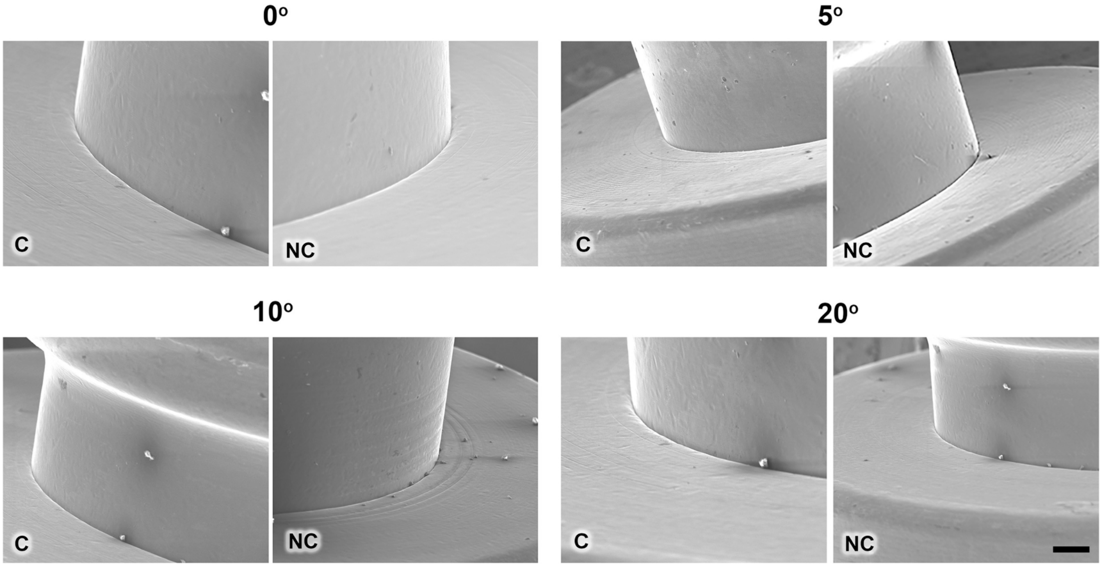

Fig. 2. Analysis of the implant and prosthetic abutment interface, under the different studied conditions. C= cycled, NC= non-cycled, Bar = 100 μm.

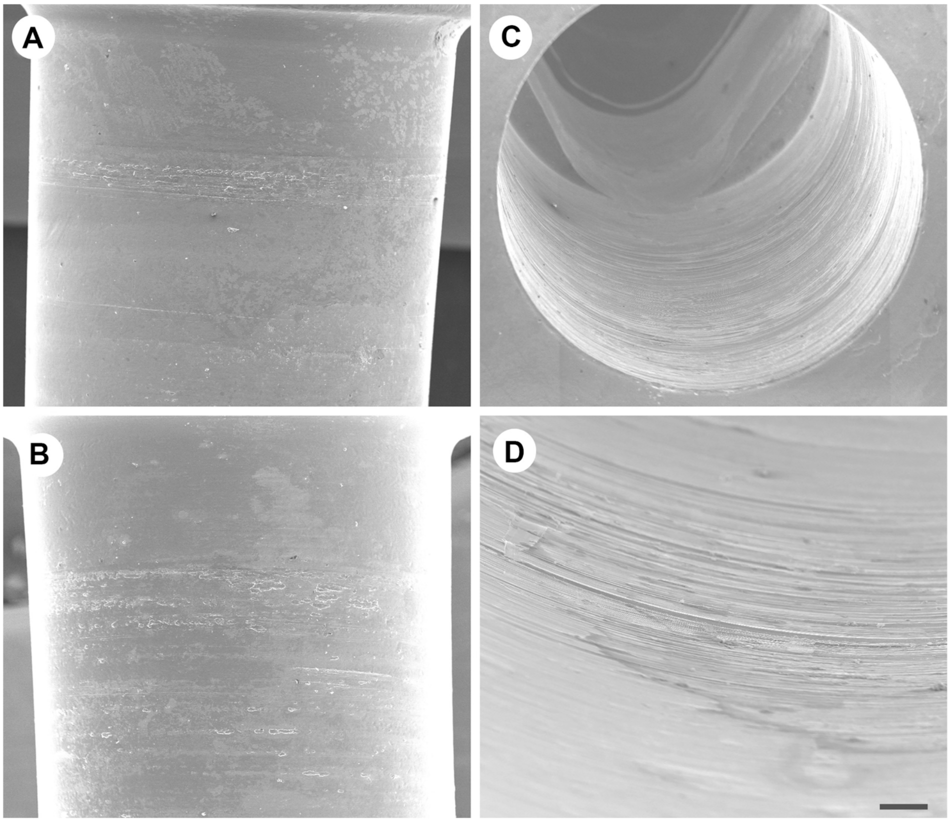

Fig. 3. Microstructural analysis of the prosthetic abutment (A, B) and internal surface of the implant (C and D), showing the presence of horizontal grooves along the long axis. Bar: A, B e C = 100 μm; D = 239 μm.

(2018) analyzed the success of implants coupled with straight and angled prosthetic abutments, and concluded that angled prosthetic abutments can be considered an adequate restoration option when the implants are not placed in ideal axial positions. The implant with frictional component used in this study allows personalized angulation of the prosthetic component up to 20°, customized prosthetic solutions. They are made of biocompatible stainless steel (18Cr14Ni2,5Mo, ASTM F138-13a) and accompanied by an angulation device that allows amplifying the manual force in a set of levers, overcoming the mechanical resistance of the prosthetic abutments, printing plastic deformations.

Morse connection has stability principle of correct adaptation between the male cone present in the prosthetic component and the female cone present in the internal face of the implant, being able to adapt perfectly through friction or juxtaposition. Hexagonal connection designs without frictional retention devices have been associated, over the years, with recurring mechanical and biological problems. Among these, literature highlights loosening of prosthetic screws and abutments and contamination of the implant as the most frequent complications (Gratton et al., 2001; Elias et al., 2006, Choi et al., 2009; Coppedê et al., 2009; Arnetzl et al. 2015). Implant-abutment connection can be considered one of the most important factors that contribute to bone loss. Several systems demonstrate the presence of gaps at the interface, even those with internal conical connection, causing alveolar bone loss (O'Mahony et al., 2000; Dibart et al., 2005; Quirynen et al., 2006). In the present study, a frictional abutment with an angled prosthetic system was used, showing its efficiency in biological sealing at the implant-prosthetic abutment interface, solving possible future complications with regards to implant contamination and the development of *peri*-implant disease.

Custom angulation of the prosthetic abutment in the system used causes an increase of tension flow in the neck region of the prosthetic abutment and, consequently, the hardening due to deformation, which together with the implant design that emulates a single body element due to the frictional connection between implant and prosthetic component, offer increased system resistance. Hardening by deformation (strain-hardening or work-hardening) is the most used among hardening mechanisms, since practically any metal or alloy can be subjected to this type of hardening (Li et al., 2014). Hardening mechanisms are ways to increase mechanical strength of a material, that is, they are ways to prevent the occurrence of plastic deformation. As in metals and alloys, plastic deformation occurs predominantly due to the movement of discrepancies, increasing the mechanical resistance means making discrepancies movements difficult. These discrepancies generate forces between the metal atoms, which increases the mechanical resistance, preventing micro-movements of the prosthetic abutment in the studied implant system. Such a condition is possible because, unlike the implant that is made of titanium, the prosthetic abutment of this system brings an unprecedented condition, its stainless-steel composition. It has a sacrifice ring causing it to harden when angled, which can be a condition for an increase in the mechanical strength of this component, decreasing its chances of suffering some plastic deformation or micro movements when in function, which can contribute to the effective biological sealing found.

Thus, in view of the different composition material of the implant and prosthetic abutment, and system angulations, this study aimed to evaluate the sealing at the implant/abutment interface, when subjected to mechanical loads simulating approximately 6 months of chewing. Results showed that in all studied conditions there was a complete microbiological sealing, which led to acceptance of the first null hypothesis. The tight frictional contact between the internal surface of the implant and the external surface of the prosthetic abutment allows a hermetic sealing in morse taper systems (Boskaya and Muftu, 2005; Aguirrebeitia et al., 2013). In fact, the small taper angle (1.50°) with a longer contact length promotes an increasing of the friction between the parts, assuring a movement-free system during the masticatory function (Mangano and Bartolucci, 2001), even with different angles frictional prosthetic abutments.

In this study, the activations were applied through the impact produced by the hammer body on the long axis of the implant and according to the manufacturer, who recommends 3 activations. This device, positioned parallel to the trajectory of the instrument impact body, standardized the strokes, avoiding variations in positioning that would cause low frictional retention, allowing an intimate contact between surfaces and juxtaposition, until there is no more displacement or friction (Zielack et al., 2011; Aguirrebeitia et al., 2013).

Since chewing movements provide more significant intrusion forces than those of extrusion and laterality, a continuous activation of the frictional abutments, without screws, could still occur over time, simply by use, which would ensure the mechanical integrity of the junction (Zielack et al., 2011). In this respect, although a wedge effect can be attributed to Morse taper connections after loading cycles, the results demonstrated a decrease in the removal force with increasing angulation of the prosthetic abutment under mechanical cycling, especially at 10 and 20° of prosthetic angulation. This data led us to reject the second null hypothesis. Abutment angulation can disrupt the maintenance of the system, favoring an increase on stress at implant, abutment and alveolar bone, which can be potentiated by different force vectors during cyclic loading (Pintinha et al., 2013; Alves et al., 2016; Hein et al., 2021). Nevertheless, implants with screwless systems, when the solid columns receive activation torque, sedimentation or relaxation effect may have occurred, in which plastic deformation of the micro-corrugations present on the machined surfaces, internal implant threads and external screw, is able to reduce the pre-load, even if the assembly is not subject to additional forces (Coppedê et al., 2009). In fact, these findings were observed in our studied groups, since a complete sealing of the prosthetic abutment and implant interface was observed.

Results of *in vitro* studies and numerical models suggest mechanical superiority of implant/prosthetic abutment connections that incorporated internal conical juxtaposition to the design of their abutments in relation to conventional screw joints regarding mechanical stability (micromovement) and flexural strength, in addition to decrease the possibility of forming micro-spaces after their initial activation (Siamos et al., 2002; Quek et al., 2006; Mangano et al., 2009; Shim et al., 2015). However, *in vivo* studies demonstrate that, despite the stability offered by these connections, they are not completely free from mechanical complications in long-term clinical use, such as loosening and fractures of prosthetic screws and abutments and biological contamination (O'Mahony et al., 2000; Shim and Yang et al., 2015). Implants with frictional abutments such as those used in the studies demonstrate a more juxtaposed contact avoiding internal contamination of the prosthetic implant-abutment interface, regardless of the angle used.

Technically, contact surfaces morphology of implants and prosthetic abutments should have little roughness or a polished finish, but most of the time it has irregularities, resulting from the machining process due to critical tolerance of manufacturing parts (Semper et al., 2010; Rack et al., 2013). The third null hypothesis was accepted in the present study, since longitudinal grooves were found in all specimens. Wear areas are commonly observed in the internal compartment of the implant as well as on the surface of the prosthetic abutment, which can be attributed beyond the manufacturing process, during the activation process, which may have contributed to the increase of the effective biological sealing.

In conclusion, the study demonstrated that regardless the decline in the removal force after mechanical loading with increasing of prosthetic abutment angulation, the frictional implant system revealed effective biological sealing preventing biological contamination, highlighting the juxtaposed contact between the implant and prosthetic abutment. Considering the harmful environment of the oral cavity, including thermal changes, humidity, as well as pH challenge, clinical studies should be performed to evaluate the maintenance of the system with abutment inclination over a long time in function.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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