



IN VITRO EVALUATION OF THE EFFECT OF SELANT APPLICATION AT THE INTERFACE OF A SCREWED TAPERED CONNECTION IMPLANT: MICROSCOPIC, MECHANICAL AND MICROBIOLOGICAL STUDY

Avaliação in vitro do efeito da aplicação de um selante na interface de um implante de conexão cônica parafusado: estudo microscópico, mecânico e microbiológico

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ABSTRACT

The objective of this in vitro study was to assess the effect of sealant at the interface of a screwed conical connection implant, regarding its mechanical behavior and bacterial infiltration. For this purpose, 60 conical implant-abutment sets were selected and divided into four groups (n=15): Group A, submitted to mechanical cycling without sealant; Group B, submitted to mechanical cycling with sealant; Group C, without mechanical cycling but with sealant; Group D, without mechanical cycling or sealant, but it was analyzed with scanning electron microscopy (SEM). The mechanical cycling test was performed with 500,000 cycles per sample with a load of 120 N and 2 Hz frequency at an angle of 30°. For microbiological analysis, 10 sets of each group (with mechanical cycling or not), were immersed in suspension containing *Escherichia coli*, and incubated at 37°C. After 14 days, the abutments were separated from the implants, the detorque forces were recorded and the presence of bacterial penetration was assessed. The remaining 5 sets of each group were cut longitudinally and analyzed, using SEM, at three points in the region of the implant-abutment interface. Mann Whitney tests were used for statistical calculations for comparisons between groups with and without sealant and between groups with and without cycling, and the paired samples Wilcoxon test for comparisons between distances. The analysis of bacterial growth was performed using Fisher's exact test. The analyzes were performed using the R and SAS program, with a significance level of 5%. The results showed that at a distance of 500 µ, in the presence of sealant, there was a greater misfit with cycling than without cycling ($p < 0.05$). In the absence of cycling there was a greater misfit without sealant than with sealant ($p < 0.05$), in both distances. There was no significant difference between the distances regarding maladjustment ($p > 0.05$). There was no growth of *Escherichia coli* on the external part in the four groups. The group without sealant and with mechanical cycling showed significantly higher internal bacterial growth than the other groups (40% of the samples), $p < 0.05$.

Keywords: Tapered Connection; Mechanical Cycling; Bacterial Sealing.

RESUMO

O objetivo deste estudo in vitro foi avaliar efeito da aplicação de um selante na interface de um implante de conexão cônica parafusado quanto ao seu comportamento mecânico e infiltração bacteriana. Foram selecionados 60 conjuntos implante/pilar de conexão cônica e divididos em quatro grupos (n=15): Grupo A, submetido a ciclagem mecânica sem selante; Grupo B, submetido a ciclagem mecânica com selante; Grupo C, não foi submetido a ciclagem mecânica mas foi utilizado o selante; Grupo D, não foi submetido a ciclagem mecânica nem utilizado selante, mas foi analisado com microscopia eletrônica de varredura (MEV). O processo de ciclagem mecânica foi realizado com 500.000 ciclos por amostra com carga de 120 N e 2 Hz de frequência em um ângulo de 30°. Para análise microbiológica, 10 conjuntos de cada grupo (com ciclagem mecânica ou não), foram imersos em suspensão contendo *Escherichia coli*, e incubados a 37°C. Após 14 dias, os pilares foram separados dos respectivos implantes, registradas as forças de destorque e avaliada a presença de penetração bacteriana. Os 5 conjuntos restantes de cada grupo foram cortados longitudinalmente e analisados, por meio de MEV, em três pontos da região de interface implante/pilar. Para os cálculos estatísticos foram utilizados os testes de Mann Whitney para as comparações entre os grupos com e sem selante e entre os grupos com e sem ciclagem e o teste de Wilcoxon pareado para as comparações entre as distâncias. A análise do crescimento bacteriano foi realizada pelo teste Exato de Fisher. As análises foram realizadas com auxílio do programa R e SAS, tendo sido adotado o nível de significância de 5%. Os resultados mostraram que na distância de 500 µ, na presença de selante, houve maior desadaptação com ciclagem do que sem ciclagem ($p < 0,05$). Na ausência de ciclagem houve maior desadaptação sem selante do que com selante ($p < 0,05$), nas duas distâncias. Não houve diferença significativa entre as distâncias quanto a desadaptação ($p > 0,05$). Não houve crescimento de *Escherichia coli* na parte externa nos quatro grupos. O grupo sem selante e com ciclagem mecânica apresentou crescimento bacteriano interno significativamente maior que os demais grupos (40% das amostras), $p < 0,05$.

Palavras-chave: Encaixe Cônico; Ciclagem Mecânica; Selamento Bacteriano.

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INTRODUCTION

Based on results obtained in experimental studies by Branemark and co-workers in the 1970s, dental implants became a scientifically proven and accepted therapy in the dental world ^{1,2}.

One of the factors contributing to the initial success of implants was osseointegration ^{3,4,5,6}, thus, misfit between the osseointegrated implant platform and its respective prosthetic abutment represents a risk for biomechanical rehabilitation ^{7,8}.

Failure to adapt these components can result in the loosening or fracture of the prosthesis screw or, even, in the fracture of the implant itself ^{7,8,9}. Furthermore, the presence of microgap may allow microorganisms present in the oral microbiota to contaminate an internal portion of the implant, which can cause an inflammation of the peri-implant tissue ^{4,10,11,12} that if untreated can lead to loss of osseointegration, bone loss, and consequently, implant failure ^{11,13,14}.

Bacterial colonization and biofilm maturation depend on a conducive ecological environment ¹⁵. Therefore, changes in local conditions, which favor the growth of pathogenic bacteria or trigger virulence factors, such as torque between implant components or loading forces when the assembly is in function, can be seen as the actual source of peri-implant disease ^{16,17,18,19}.

Several authors have reported the presence of bacteria inside titanium implants, even in state-of-the-art, where the bidirectional passage of fluids is evident ¹². Furthermore, in the external portion of the implant, in peri-implant regions, after its exposure to the oral environment, a high prevalence of gram-negative anaerobic pathogens has been observed, in addition to inflammatory infiltrate, always present at the implant-abutment interface ^{20,21,22}.

In order to avoid the problems caused by microleakage at the prosthetic implant-abutment interface, cone-morse implants were developed. According to their manufacturers and some studies, such implants present several advantages over conventional external and internal hexagon implants, such as bacterial sealing, no gap, stable anti-rotational fixation, high mechanical stability, greater abutment strength, no screw loosening, and bone and gingival tissue stability ^{15,23,24,25}.

The success of tapered connection abutments encourages researchers and manufacturing companies to focus on understanding and evaluating its mechanical properties. Biomechanically, studies have demonstrated the superiority and better predictability of behavior of this type of connection when undergoing axial and lateral loads, remaining stable in the long term and making its mechanical strength similar to that of one-piece dental implants ^{15,26,27,28}. In addition, this has been reported as a safe, reliable connection and as an essential factor for bone crest

maintenance due to the reduction of microgap and consequent decrease in the possibility of bacterial contamination^{26,29,30,31,32}.

Although there are better connections now, research using different tapered connection systems without cycling^{33,34,35,36,37,41} have shown the existence of bacterial infiltration through the implant-abutment interface.

Thus, it is necessary to seal the existing microgaps in the connections since they are difficult to close mechanically^{6,42}. Several types of materials have been studied to enable such sealing, for instance, chlorhexidine and thymol varnish, silicone rings, 2% chlorhexidine solution, gutta-percha, and sealing silicones^{6,43,44,45,46,47}.

Products composed of siloxane, silica, and paraffin, such as Kiero Seal (Kuss Dental, Carl-Bechem, Germany), can be used as sealing material, considering its good properties, such as self-curing, biocompatible, chemically and physically inert^{6,48}.

PROPOSITION

This study aimed to evaluate whether there was bacterial infiltration in the spaces between the implant and the prosthetic abutment and the action of a sealant at this interface, under the influence of masticatory cycling, using mechanical, microbiological, and scanning electron microscopy (SEM) analysis in an implant model with a tapered connection.

METHODOLOGY

This study was approved by the Ethics and Research Committee of the São Leopoldo Mandic School, under protocol number 20210264.

This study used sixty sets of implants with tapered connection (Unitite cone morse reference UCN 3515, SIN, São Paulo, Brazil) and their respective universal prosthetic abutment (reference AISIT454008) fixed with a screw supplied by the same company in their original packaging. In order to verify a possible sterilization failure caused by the manufacturer, an implant was randomly selected and placed in a culture medium for 48 hours. After this period, there was no turbidity in the culture medium, thus proving that sterilization was effective.

The samples (n=60) were divided into four groups (n=15 implants-abutments) according to the presence or absence of sealant at the implant-abutment interface and/or mechanical cycling: Group A, under mechanical cycling, without sealant; Group B, with mechanical cycling, with sealant; Group C, without mechanical cycling, with sealant; Group D, without mechanical cycling, without sealant.

Torque application

For the preparation of the specimens, 6 bases were made, also prefabricated in brass, which allowed the fixation and assembly of the implant-abutment sets to be subsequently submitted to

cycling, according to the recommendations of ISO 14801:2012. After turning, each base received a final cylindrical configuration, measuring 2.3 cm in diameter, 2.0 cm in height on its highest side, and 1.0 cm on its lowest side, due to a surface inclination of 30°. Then, these bases were drilled to receive implants with dimensions of 13 mm in length by 3.5 mm in diameter, inclined, fixing the implants so that their central axis remained at a 30° angle in relation to the equipment loading direction.

The implants were placed in the perforations with a depth of 10 mm, kept 3 mm to the outer region of the fixation device, simulating a representative case of bone loss (ISO 14801:2012). In order to stabilize the implants, screws were placed laterally on the support faces, pressing the implant against its internal lateral wall, which provided locking without deformation.

A torque wrench recommended by the manufacturer was used for fixing the abutments to the implants, coupled with a manual torque wrench. Following the manufacturer's specifications, an insertion torque of 20 Ncm was used and subsequently measured using a digital torque wrench.

Sealant application

Following the manufacturer's recommendations, the Kiero Seal (Kuss Dental, Carl-Bechem, Germany) sealant was applied inside the implant with an applicator tip attached to the mixer. Subsequently, the abutments

were immediately connected to their respective implants, screwed with standardized torque (20 Ncm). For this purpose, a specific wrench was used, coupled to a manual torque wrench, revised by an electronic torque wrench. Then, the varnish was applied around the prosthetic abutments using a microbrush. Finally, for stabilization and fixation of the samples, a bench vise was used.

Mechanical cycling

The samples of groups A and B were submitted to mechanical cycling. The specimens were fixed to their bases and coupled to the mechanical cycling machine (Elquip®, SP, Brazil), which simulated the frequency of human masticatory cycles. A total of six sets were tested simultaneously at each cycling period so that three samples from each group were cycled at the same time.

Each implant-abutment set was submitted to mechanical cycling with a compressive load of 120 N and frequency of 2 Hz at an angle of 30° in relation to the implant long axis, following ISO 14801:2012 recommendations, and 500,000 cycles per sample were performed^{15,49}. The applied load represented the average occlusal force or masticatory load in the posterior region^{50,51,52,53} and the number of cycles simulated approximately 6 months of function^{52,54,55}.

Microbiological assessment

All procedures were performed inside a laminar flow chamber previously disinfected and

covered with a sterile field for the microbiological analysis. A suspension of *Escherichia coli* (American Type Culture Collection - ATCC 25922) was used, kept at 37°C for 18 h, under microaerophilic conditions, and then, centrifuged at 2200 rpm, at 18°C for 5 min with optical density of the suspensions adjusted to 1.0 at 540 nm, which corresponded to a microbial concentration of 12X10⁸ cells / ml.

Each group of implant-abutment sets was immersed in its respective flask, all sterile and identified, with 75 ml of *E.coli* suspension. Then, they were taken to the bacteriological oven and incubated for 14 days 10,56 at a temperature of 37°C under aerobic conditions. The observation period for possible contamination for 14 days provided a safety margin, consistent with what is recommended in the literature for initial colonization of the peri-implant sulcus 57,58. The culture medium was changed every 48 hours 59.

After the incubation period, the implant-abutment sets were carefully removed from each flask and dried on sterile absorbent paper to remove excess bacterial broth. Each sample was then washed three times in sterile distilled water and again dried on absorbent paper.

The outer surface of each sample was manually disinfected with 0.25% peracetic acid (Proxitane Alfa, Thech desinfecção Ltda, São Paulo, SP, Brazil) by mechanical rubbing 60 for 20 seconds and then dried with absorbent paper.

All samples were fixed to the support bases once more to separate the abutments from the

implants and to register the force required to the detorque. The detorque values were determined and registered using a digital electronic torque controller. The detorque values were registered and recorded in Ncm. All procedures were performed by the same operator, previously trained or adjusted, in a sterile environment.

To ensure that the external portion was not contaminated, before separation, a microbrush dampened in sterile 0.9% saline solution was used in each set, to a thorough scrubbing across the surface of the implant-abutment interface. In addition, each microbrush was immersed in a culture medium (BHI), acting as external contamination control³⁷.

Subsequently, a moist and super-fine microbrush was carefully rubbed over each implant's most apical internal surface, without touching the lateral walls, to collect bacteria that might have penetrated the interface. Each microbrush was inserted into a tube with 5 ml of sterile BHI broth, submerged, and incubated at 37°C for 48 hours.

Bacterial penetration was assessed by checking the presence of turbidity in the broth, which indicates that the sealing of the implant-abutment interface is not able to prevent bacteria passing from the culture medium to inside the implant.

From each sample with suspected contamination, aliquots of the culture medium contained in the tube (10 µl) were collected, plated on BHI agar, and incubated at 37°C

for 24 h to confirm the results of the visual macroscopic examination of bacterial growth. The gram staining method was performed for each plate. The slides were observed under an optical light microscope to confirm the growth of only Gram-negative bacilli (*E.coli*).

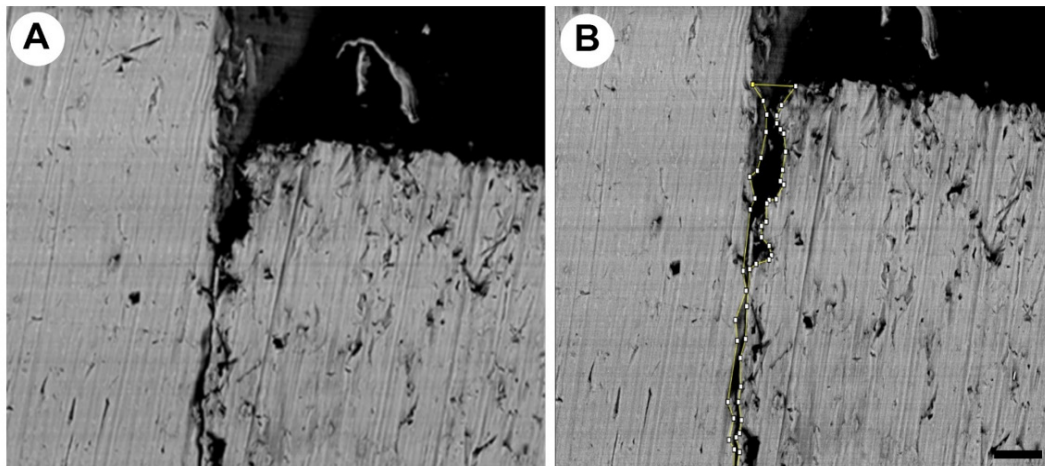
Scanning Electron Microscopy (SEM) analysis of adaptation at the implant-abutment interface

The sets were embedded in epoxy resin, producing a block for future sectioning. After curing time, the assembly was cutted longitudinally using a water-cooled cutter with

diamond disk. Then, the samples were polished using an electric sander, and 4 grit sizes. Before SEM analysis, they were cleaned using an ultrasonic tank and pressure water jets, to remove cutting and polishing debris.

The interfaces were analyzed on the most cervical surface, for an implant-abutment intersection up to 500 μm of depth and apical, and for a region from 500 to 1000 μm of depth. Using the ImageJ software (National Institute of Health), the misfit area was measured in μm^2 on both sides of the specimens. (Figure 1).

Figure 1. A: most cervical region measurement region of the implant-abutment intersection. B: area measurement using ImageJ software. Barr: 10 μm .



Source: own authorship.

The misfit area was calculated using ImageJ (National Institute of Health, USA) software, and was measured in μm^2 in the most cervical (0 to 500 μm in depth) and the most apical (500 to 1000 μm in depth) regions on both sides of the specimens.

Statistical analysis

Descriptive and exploratory analyses of the data were performed. For the misfit data, the Mann–Whitney U test was used to compare the groups with and without sealant and to compare the groups with and without cycling. The paired samples Wilcoxon matched-pairs signed rank

test was applied to compare the distances. The Fisher’s Exact test was used for microbiological analyses. Both analyses were performed using the R and SAS softwares, for a significance level of 5%.

RESULTS

Microbiological assessment

Absolute and relative frequencies of culture media with turbidity in the internal part of

the implants, in the different conditions evaluated in this study, are shown in Table 1. Turbidity was observed only in the group submitted to cycling test, without varnish, in 40% of samples (p=0.0092). Contamination was not observed in any of the other samples (p>0.05). No turbidity was found in any of the samples in the tests performed with the external collection.

Table 1. Frequency (%) of samples with *Escherichia coli* growth in relation to the presence or absence of sealant and mechanical cycling.

Sealant	Cycling	n (%)
Presence	Presence	0 (0.0%)
Absence	Presence	4 (40.0%)
Presence	Absence	0 (0.0%)
Absence	Absence	0 (0.0%)
p-value		0.0092

Source: own authorship.

Detorque force

Detorque force results are shown in Table 2. Regardless of sealant or mechanical

cycling test, there was no difference between the evaluated groups (p>0.05).

Table 2. Mean (standard deviation) of the detorque force in relation to the presence and absence of sealant and mechanical cycling.

Sealant	Cycling test	
	Presence	Absence
Presence	21.00 (4.29)	19.30 (6.57)
Absence	23.44 (5.79)	22.40 (3.98)

p(sealant)=0.1333; p(cycling test)=0.4451; p(interaction)=0.7853.

Source: own authorship.

Misfit assessment

Results of the misfit areas can be found in Table 3.

Regarding the distance of 0 to 500 µm, there was no difference between the groups with or without cycling test in relation to sealant use ($p>0.05$).

However, in the absence of cycling, a larger gap was observed in the group without

sealant than in the group with sealant ($p>0.05$).

In relation to the distance of 500 to 1000 µm in the presence of sealant, misfit was higher in the group with cycling than in the groups without it ($p<0.05$). In the absence of sealant, there was no difference in terms of gap size in the presence or absence of mechanical cycling ($p>0.05$).

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Source: own authorship.

DISCUSSION

Despite the technical and scientific progress in dental implant systems, one of the most concerning factors is the implant-abutment interface ¹⁵. A perfect adaptation between the implant and its components is vital to prevent implant loss, given the potential risk of bacterial colonization in the misfit area, leading to peri-implant pathology, which compromises osseointegration ^{10,11,17}.

The microscopic space caused by implant-abutment misfit can vary from 1 to 100

µm, and enable the infiltration of fluids and macromolecules originating from interstitial fluid and saliva. Bacterial permeability is responsible for the contamination of the implant-abutment interface, even in gaps smaller than 10 µm, which are considered cases of good marginal adaptation of components and implants, since the average size of the species in the oral microbiota varies from 0.1 to 1.5 µm in diameter and 2 to 6 µm in length ^{10,41, 59}.

Chemotactic stimulation caused by the pump mechanism of bacteria and endotoxins, resulting from micromovement, initiates and

supports the recruitment of inflammatory cells at the bone-implant interface, which leads to persistent inflammation and progression of alveolar bone loss^{19,35,63,64}.

The geometric design of a conic connexion might not seal the parts' interface, but it can reduce, or prevent, the micromovement of prosthetic components, by the frictional locking of the parts inside the implants^{11,25,65}. Biomechanical complications can then be avoided^{7,66} and a hermetic seal to bacterial penetration can be provided^{38,67,68}. Some studies point that even if the parts are adapted, conical connections will still be contaminated^{41,69}. The results of this study did show that the contamination in the group submitted to mechanical cycling test without sealant could be explained by bacterial infiltration through longitudinal surface defects or fitting failures. Although locking was obtained, the contact area between the surfaces was not completely effective in promoting sealing and avoiding bacterial infiltration.

Other factors such as masticatory loading and the detorque of the prosthetic abutment can reduce abutment stability, enabling bacteria penetration through the microgaps^{15,19,70}. Therefore, sealing the microgap of the implant-abutment interface is a challenge for implant dentistry^{71,72}.

Many materials have been used as barriers, chemical or otherwise^{6,70}. Some studies have shown that the use of sealant reduced contamination, although none of them was capable of completely sealing the implant-abutment junction^{43,44,46,73}.

Kiero Seal material was chosen to form a mechanical barrier, since it is a chemically and physically inert material, without bacteriostatic and or bactericidal⁷⁴. The groups with sealant showed complete effectiveness of the interface sealing, regardless of mechanical cycling.

Additionally, Kiero Seal did not prevent connection reversibility, as do thread locker adhesives sealants, thus keeping the prosthetic abutment removable in case of need. Although this study's results are relevant, some limitations were noted, such as the mechanical cycling test, the fact that the bacterial penetration test was performed under static conditions, and the lack of torque variation.

This study adopted criteria consistent with scientific work variables, including the influence of mechanical action in the stability of the structures, and for this purpose, mechanical cycling testing was crucial to simulate oral conditions⁷⁵. Mechanical cycling test is an accepted experimental model of mechanical fatigue testing to reproduce unfavorable oral environment and has been used widely to test the influence of loading on implant-supported prostheses and their prosthetic components^{76,77,78}.

According to the Academy of Dental Material (ADM guidance), the benefit of constant load cycle fatigue tests is that they can explain cyclic effects on stiffness and stability of prosthetic materials, in addition to being more relevant to simulate real conditions, increasing the significance of in vitro studies⁷⁸.

CONCLUSION

Despite the favourable results, and although the sealant has shown to be effective to the control of bacterial infection, it is crucial to provide clinical control of implants with conical connection, with attentive guidance to periodical visits of patients for clinical and x-ray evaluation, as well as continuous supportive periodontal therapy to prevent and reduce the risk of infection of peri-implant tissues.

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Observação: os/(as) autores/(as) declaram não existir conflitos de interesses de qualquer natureza.