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Static Bacterial Leakage in Different Conometric Connections: An In Vitro Study

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Abstract: This in vitro study aims to evaluate the bacterial microleakage of three conometric connections. Sixty dental implants (3P implafavourite Scalenghe) were divided in groups (n = 20): Cone–Morse with passing screw (Group 1); Cone–Morse with solid abutment (Group 2); and Conometric connection with esthetic abutment (Group 3). The implants were fixed in resin bases. Then, 1.0 μL of *Streptococcus oralis (SO)* was inoculated in the internal platform in 10 fixtures for each group, and another 10 were inoculated with *Pseudomonas aeruginosa (PA).* The abutments were then screwed, and five implants from each subgroup were randomly selected for SEM inspection to ensure that the abutments were installed correctly. Data were submitted to statistical analysis, ANOVA and Fisher's Least Significant Difference ($p \leq 0.05$). The turbidity of the broth was monitored for 14 days of follow-up in order to determine the penetration of the bacterial suspension into the surrounding solution, but the observation of the samples lasted until the 90th day, in which there was no difference between the two. Microbial contamination was found in 30%, 20%, and 50% of Group 1, Group 2, and Group 3, respectively, but there were no statistically significant differences between the groups, and PA showed greater infiltration than SO. Although no statistically significant differences were found, cone morse connections showed lower infiltration percentages, respective to the conometric connection with 18° angle.

Keywords: bacterial leakage; Cone-Morse connections; implant; implant-abutment connections

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1. Introduction

Biological factors such as aging, social, and cultural, as well as genetic, trauma, and even iatrogenic factors, lead to tooth loss that requires functional, aesthetic, and phonetic rehabilitation to restore the individual's health [1,2].

In addition to single prostheses or conventional prosthetic appliances, osseointegrated implants can be used, which can also offer masticatory stability with the retention of rehabilitations in the stomatignant system. However, in addition to the stability obtained with osseointegration, implants, usually with at least three components, can also be lost even with a high success rate that reaches 97% of cases [3].

Failure in prosthetic rehabilitation treatment may denote a number of factors, one of the most complicated of which is bone loss around the dental implant. Factors such as fixture and abutment design, surgical trauma, occlusal overload, peri-implantitis, autoimmune host reaction, and bacterial microleakage contribute to bone and implant loss [2,4,5].

Implant-abutment microleakage has been studied since the 1990s and refers to bacterial microleakage between the interface of the pieces, caused by the presence of a microgap [6]. The size of this microgap can range from 1 to 49 μm, according to the different connections. The passage of bacteria and endotoxins can occur freely through the microspace between the connections, since the size of the microorganisms can vary from nanometer to a few micrometers, depending on the type [4,6–8].

Although one-piece dental implants showed encouraging biological results, two-piece implants continue to be the preferred method because they are also easy to manage in the prosthetic phase [9]. A microgap between the abutment and the implant as well as between the dental prosthesis and the abutment seems inevitable for two-part implant systems, despite the fact that both are tightly connected with one-piece implants [10].

The formation of these microgaps and the presence of bacteria may be responsible for peri-implant inflammation and consequent bone loss adjacent to the implant-abutment [11,12]. As a result, internal and marginal adaptation at the implant-abutment interface are important aspects because they are directly connected to biological integrity, structural stiffness, and the maintenance of healthy peri-implant tissue [13]. Therefore, the relationship between the geometry of the implant-abutment connection can have a great influence on microgap formation and microbial infiltration. Yet, according to the literature, Morse–Cone connections usually have low levels of contamination compared to other geometries [4,14,15]. An important factor is represented by the rotational tolerance that determines the stability of the implant/abutment connection [16,17].

The connections currently found are internal hexagon (IH) and external hexagon (EH). It has been shown that if the rotational tolerance of EH is less than 2 degrees, it could resist to a mean of 6.7-million loading cycles before loosening, while if this angle is greater than 5°, there will be a reduction of 67% to the resistance of screw-loosening [17].

Based on the geometric conformation of the inner part of the fixture platform, internal hexagon can be further classified in clearance-fit, conical, and combined connections [18].

In order to reduce friction between the components, clearance-fit connectors include parallel walls on the interior of the fixture and the abutment with a small gap. To prevent the rotation of the prosthetic components, geometric features called "index" of different shapes can be added to these connections, with an anti-rotational function [18].

In conical connection, the inner part of the implant's platform has a conical shape that creates a tapered interface with the corresponding portion of the abutment without the presence of an inner space.

Anti-rotational geometric characteristics can also be introduced at the apical region of the abutments in these connections [18]. Morse-taper connections were introduced by Stephen Morse in 1864 for industrial machines and in the 1990s in implant dentistry, with an original angle inclination of 2°50′. However, today on the market, Morse-taper connections can reach until 18°, although an acceptable interference happens for taper angles smaller than 5.8° [17,19]. So, to simplify the text, we will refer in the "manuscript" to the terms "Cone–Morse," "taper Morse," and "conical connection" without distinction, but in each case, we will specify the degree of the connection.

Conical connections are the most often used connectors for dental implants and abutments nowadays, as they provide increased mechanical stability under loading, minimizing abutment loss [14,20,21]. This hypothesis – that the Morse taper is a fitting design that employs friction to embed one cone into another cone with an angulation between two and four degrees without the use of screws, creating a cold weld between the components - is supported by Peruzetto et al. [22].

In recent times, the conometric system has been proposed for use in fixed prosthesis connections, performing satisfactory results in rehabilitations [10,23]. Such a system consists of a conical coping attached to the prosthesis and inserted into a conical abutment, and when an adequate insertion force is applied, this system can provide an effective fixed connection [10]. According to Gehrke et al. [13], the conometric system uses a wedge effect to achieve its high sealing and retention efficiency.

Nonetheless, despite demonstrating hermetic contact, absolute congruence between the implant and the abutment, and high-leakage resistance, evidence of bacterial infiltration, was observed within the implant cavity, even on Cone–Morse connections [3,21,24,25].

Cone–Morse implants are used in single, partial, or total oral rehabilitations due to their undeniable benefits and clinical longevity. There are several brands and models available to the clinician. The aim of this in vitro study was to evaluate the bacterial microleakage of three different types of conical connections: an abutment with a passing screw (Cone–Morse connection), a solid abutment (Cone–Morse connection), and a conometric connection with an esthetic abutment. In particular, bacterial microleakage of two bacterial species into the three implant connections was evaluated over a period of 14 and 90 days.

2. Materials and Methods

The sample size was calculated by using the analytical package G*Power 3.1 (Heinrich-Heine-Universität Düsseldorf, Germany). A total of 60 dental implants (3P implafavourite Scalenghe-Torino–Italia) with a diameter of 3.30 × length 10.0 mm was used in this in vitro study and divided into three groups:

- Twenty Cone Morse 2° (1° each side) → Abutment with passing screw (Group 1);
- Twenty Cone Morse 2° (1° each side) → Solid abutment (Group 2);
- Twenty Conometric connection 18° (9° each side) → Esthetic abutment (Group 3).

In order to better understand the study, below is the diagram of the experiment (Figure 1) and the schemes of the different implant-abutment connections used (Figures 2 to 4).

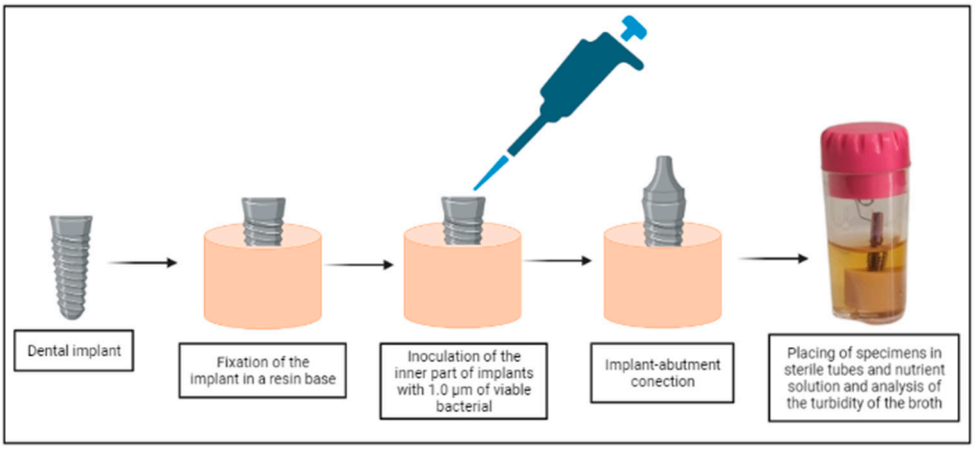


Figure 1. Experiment diagram.

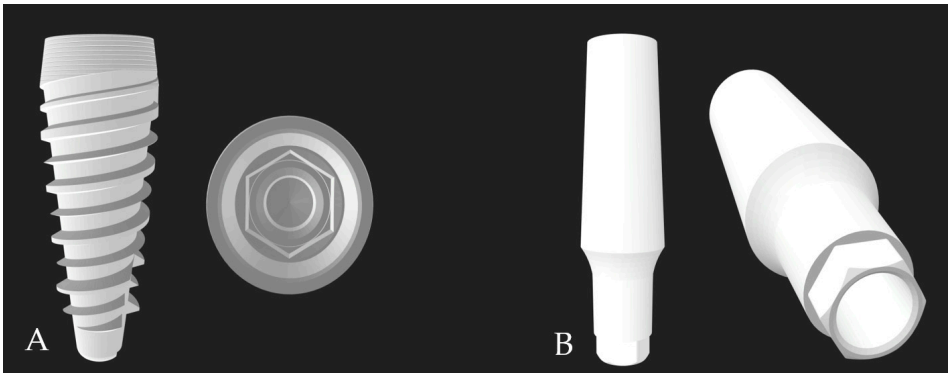


Figure 2. (**A**) Cone Morse 2° (1° each side); (**B**) Abutment with passing screw.

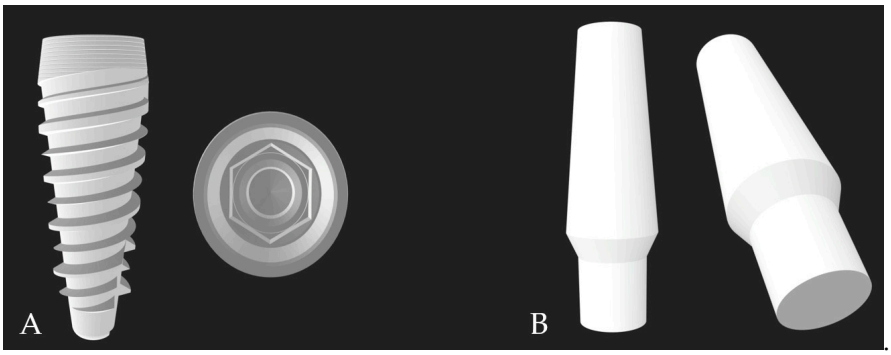


Figure 3. (**A**) Cone Morse 2° (1° each side); (**B**) Solid abutment.

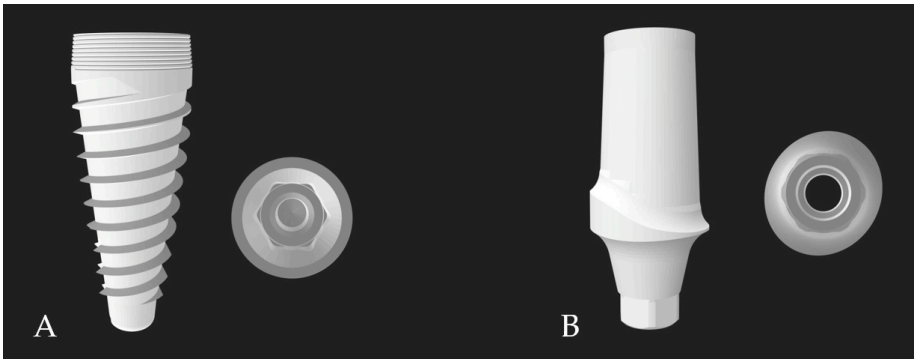


Figure 4. (**A**) Conometric connection 18° (9° each side); (**B**) Esthetic abutment.

The implants and abutment connections used were sterile samples; all other study materials were sterilized in an autoclave, and all laboratory procedures were performed under laminar flow in absolute sterility.

Subsequently, the implants were fixed on photopolymer resin bases (SprintRay Inc.–Los Angeles, CA, USA) to mimic the primary stability with the aid of a device (Figure 5).

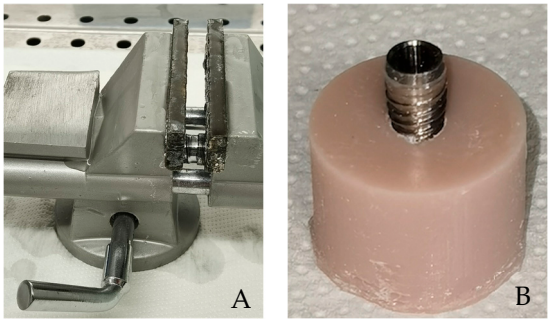


Figure 5. Implants fixation (**A**) Device used to fix the base with the implant; (**B**) Implant fixation on resin base.

2.1. Bacterial Inoculation

Pure cultures of *Streptococcus oralis* CH 05 and *Pseudomonas aeruginosa* ATCC 15442 were used for implant inoculation. The investigated microorganisms *S. oralis* and *P. aeruginosa* were initially plated over fresh trypticase soya and cetrimide agar, respectively. These plates were then incubated for 24 h at 37 °C and standardized at an optical density OD600 0.125 [4,20,26]. The microbiological experiment included 10 samples from each group.

The groups were divided into two subgroups (n = 10) in which 10 fixtures were inoculated with 1.0 µL of standardized broth culture with oral *Streptococcus oralis* CH 05 (SO),

and the other 10 were inoculated with *Pseudomonas aeruginosa* ATCC 15442 (PA) (Figure 6).

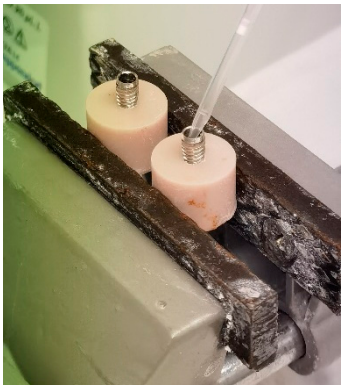


Figure 6. Inoculation of implant's inside with 1.0 μL of viable bacterial.

The quantity of bacterial broth, to inoculate inside the implant platform, was previously evaluated with a preliminary test.

2.2. Abutment Connection

After inoculation of the implant, the abutments were screwed at specific torques, following the Manufacturer's indications:

- Cone–Morse 2° (1° each side) → Abutment with passing screw: 35 Ncm;
- Cone–Morse 2° (1° each side) → Solid abutment: was fixed to it by the percussion of a beaver compatible with the platform of the abutment with 200 gr of force (Figures 7 and 8);
- Conometric connection 18° (9° each side) → Esthetic abutment: 25 Ncm.

Figure 7. Fixation of group 2 "solid abutment"by using a special key percussed with a force of 200 gr.

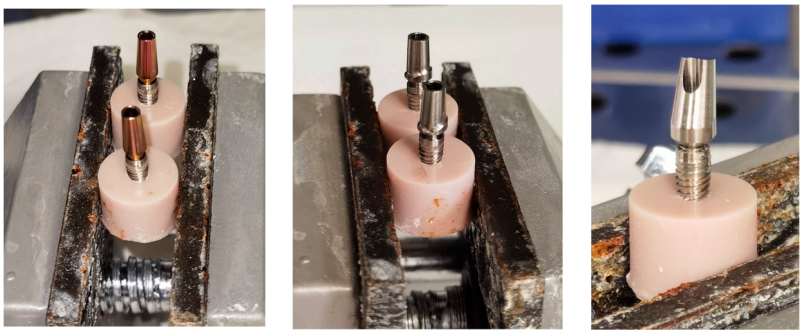


Figure 8. The three connections tested.

Two experimental tubes were used with nutritive solution and inoculated with 1.0 uL of *S. oralis* and *P. aeruginosa*, respectively, as a positive control. They demonstrated bacterial growth in response to the solution's turbulence, which supported the viability of the microorganisms throughout the experiment. Two experiment tubes that contained only a sterile nutrient solution were used as a negative control. This was supported by the transparency of the solution and conventional microbial culture techniques.

After the inoculation and abutment connection, the assembled components were completely submerged in the nutrient solution for 1 min while being rolled around to check for inadvertent contamination of the exterior surface. Following an assessment of bacterial growth in plates, tubes with cloudy broth (representing colonization/contamination of the implant's exterior sections) were disqualified from further investigation. The quantity of nutritional solution needed in the test vials was then precisely calculated for each implant system after the specimens were put into sterile tubes, keeping the fluid level just above the implant-abutment (I-A) interfaces (Figure 9A). Following that, a cap was placed on each tube, and it was then left for observation (Figure 9B).

(**A**) (**B**)

Figure 9. (**A**) Placing of specimens in sterile tubes and nutrient solution; (**B**) Closure of tubes with cap.

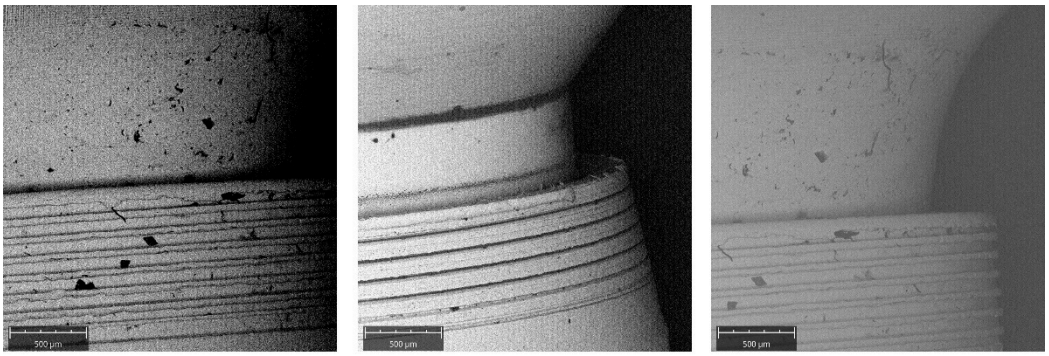
The test tubes used as external contamination controls, positive control tubes, and negative control tubes, as well as all of the vials containing the assemblies, were incubated at 37 °C under aerobic conditions. The culture broth in the vials containing the assemblies was changed every four days during their maintenance period of 14 days. The visual assessment of the broth's turbidity allowed for the determination of any potential bacterial suspension infiltration into the surrounding solution (Figure 10). Every day, the samples were examined and the presence or absence of turbidity was noted. Such leakage caused bacterial colonization and resulted in a cloudy solution. In order to confirm the purity of the microorganism that had been inoculated in the inner part of the implant, and to determine the presence of *S. oralis* and *P. aeruginosa*, 1 uL of the cloudy solution was analyzed with a gram stain and by colony morphology in trypticase agar plates (for *S. oralis*) and cetrimide agar (for *P. aeruginosa*), incubated at 37 °C for 24 h.

The results of 14-days follow-up were confirmed also after 90 days of observation.

Figure 10. Samples placed into the nutrient solution during the follow-up. **Right** = turbidity of the broth as a sign of bacterial penetration; **Left** = no contamination.

2.3. SEM Analysis

In order to confirm the proper fit of the abutments, five implants for each subgroup were examined at SEM at 240× of magnification at 15 Kv [4]. These images were used as a means of qualitatively confirming the perfect fit of the prefabricated components (Figure 11). Therefore, in order to reduce the possibility of microbial contamination during the SEM observation, the samples were decontaminated, disinfected, and sterilized before the analysis.



(A) (B) (C)

Figure 11. (**A**) SEM image of the Cone Morse 2°—Abutment with passing screw (**B**) Cone Morse 2°—Solid abutments (**C**) SEM image of the Conometric connection 18°.

The SEM analysis was performed using a Phenom ProX scanning electron microscope (Phenom-World BV, Eindhoven, The Netherlands) with the Element Identification (EID) software (Phenom ProSuite Software, Phenom-World B.V., Eindhoven, The Netherlands). The parameters used for SEM observation were: 15 kV, Map detector, BSD full.

2.4. Statistical Analysis

Results were recorded dichotomically as "infiltrated" and "not infiltrated." The statistical software used SPSS Statistics for Windows, Version 21 (IBM SPSS Inc., Chicago, IL, USA). The average number of infiltrated connections and the standard deviation were calculated. The Levene test was used to examine the homogeneity of the groups: For homogeneus variables, the ANOVA and LSD Test was performed; in the case of no-homogeneus variables, the Kruskal–Wallis test was calculated. Microsoft Office 365 (Redmond, Washington, USA) was used to produce the graph of results and to calculate the percentage (%) of infiltrated connections by using the following formula: (average number of infiltrated connections of each group*100)/ number of connections examined for each group.

3. Results

The connections' implant-abutment were correctly fitted, according to all SEM observations (Figure 11).

The results of bacterial microleakage at the 14th day were also confirmed at the 90th. Consequently, the results are presented once (Figure 12).

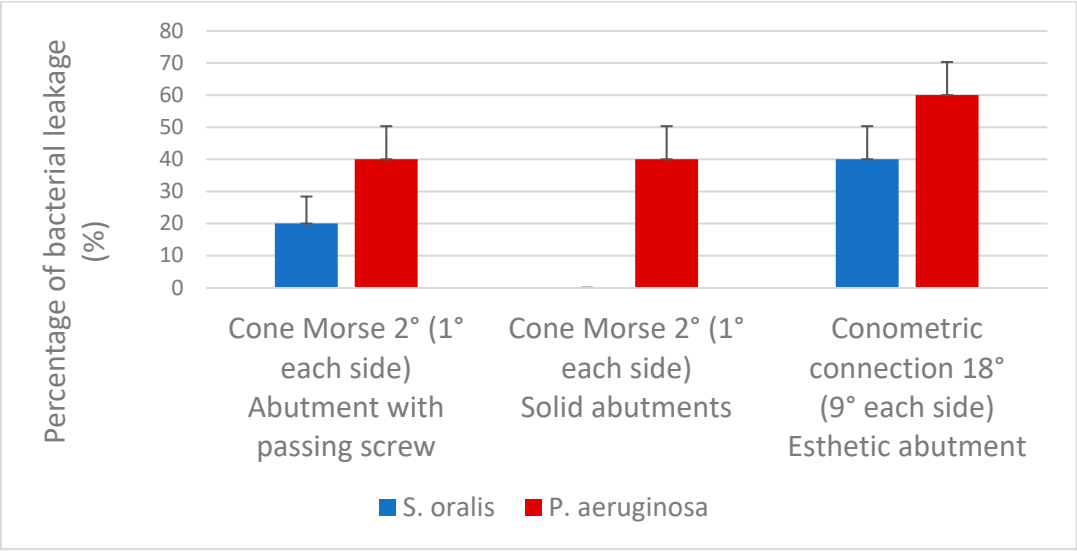


Figure 12. Percentage of bacterial leakage in the three different conometric connections over 90-day observation period (Error bars = standard deviation).

The percentages of mean total bacterial contamination during the observation period were 30%, 20%, and 50%, for Groups 1, 2, and 3, respectively. The Levene test showed a lack of homogeneity between the variables ($p < 0.001$), so the Kruskal–Wallis test was used; it showed the lack of statistically significant differences among the groups ($p = =0.055$).

According to the results, it was possible to verify that the bacteria behaved differently, in which *Streptococcus oralis* presented a lower contamination capacity in relation to *Pseudomonas aeruginosa* that more infiltrated in all implant connections.

In particular, in Group 1 (Cone Morse 2°-Abutment with passing screw), 20% bacterial contamination was found in I-A assemblies seeded with *S. oralis* and 40% seeded with *P. aeruginosa*. In Group 2 (Cone Morse 2°-Solid abutments), no bacterial contamination was found in I-A assemblies seeded with *S. oralis,* and 40% was found in those seeded with *P. aeruginosa*. In Group 3, meanwhile, I-A assemblies seeded with *P. aeruginosa* showed a 60% bacterial infiltration, whereas 40% was determined for *S. oralis*.

In addition, concerning the data organized for the different bacteria used for infiltration, the Levene tests showed that data were not homogeneus ($p < 0.001$), so the Kruskal–Wallis test was performed; it showed statistically significant differences among the groups ($p = 0.002$). For all the connections tested, the *P. aeruginosa* showed a significant capacity of bacterial infiltration in respect to *S. oralis.*

4. Discussion

The presence of a gap at the interface between implant and abutment represents the first step of bacterial colonization and then biofilm consolidation, with the activation of an inflammatory infiltrate that could trigger marginal bone loss. The performance of the implant-abutment interface is of crucial importance for the long-lasting soft and hard peri-implant tissues. Consequently, over the years, a tremendous number of different connections have been produced to reach the objective of "zero infiltration." Many factors have been considered fundamental for the bacterial accumulation at the implant-abutment interface, like the type of connection, the contact area, the angle of the conometric connection, the presence of an antirotational system, and the materials used.

In this study, for the first time, the evaluation of the bacterial leakage of three different conometric connections, two Cone–Morse, and one 18° of the same manufacturer with the outgrowth model [18] was performed. Through this classic methodology, it is possible to verify bacterial penetration of the implant-abutment connection.

Although no significant differences have been found among the groups, Cone–Morse connections with solid abutments showed lower bacterial infiltration. In particular, samples of Group 2 seeded with *S. oralis* showed no infiltration. On the contrary, *P. aeruginosa* presented a higher capacity to infiltrate all the connections.

Bacteria such as *Streptococcus oralis* and *Pseudomonas aeruginosa* can be easily found in situations like this, as they are highly abundant at implant sites and periodontal infections and may form biofilms and result in peri-implantitis lesions [20,26–29].

In this in vitro study, it was observed that the passage of *P. aeruginosa* occurred in all groups, contrary to *S. oralis,* which was unable to infiltrate all the connections, confirming the results obtained in a previous study by D'Ercole et al. [4].

It is important to emphasize that *P. aeruginosa* is a Gram-negative aerobic motile rod with dimensions ranging in size from 0.5 to 0.8 μm by 1.5 to 3.0 μm [30]. Almost all strains move using a single polar flagellum. However, some have two or three flagella. It can be highly pathogen and also resistant to many commonly used antibiotics, low concentrations of antiseptics, dyes, and high concentrations of salt [30]. On the contrary, *S. oralis* is a Gram-positive facultative anaerobe spherical coccus with a diameter of 1–3 μm. The ability of bacteria to pass the implant/abutment connection is not a consequence of being an aerobe, anaerobe, or facultative one, and it is not directly correlated with pathogeny. In our previous study, we compared the bacterial leakage of different connections by using *P. aeruginosa* suspension and *Aggregatibacter actinomycetemcomitans*. The latter is anaerobe facultative and showed a lower ability of bacterial infiltration, in respect to the aerobe *P. aeruginosa* [2,31].

Although there are some little differences among the percentage of mean total infiltration, no significant differences have been found among the three types of connections. The percentage of total bacterial infiltration was comprised between the 20 and the 50%, and the results are totally in line with previous work of D'Ercole et al. [4] and in the study of Aloise et al. [32], in which 20% of bacterial infiltration in the Cone–Morse group was determined.

In our previous studies, in which the same methodology was applied, none or little bacterial leakage was observed in Cone–Morse connections compared to other connections [4,5,20,28,29,32]. These results have been supported by the literature [14,15,20,21,33,34]. Teixeira et al. 2011 [3] conducted one of the first studies to demonstrate the passage of microorganisms through microgaps in the Cone–Morse system, shedding light on the failure of cold welding. Even so, it is possible to notice that the Cone–Morse connection presents less bacterial infiltration compared to the classic internal and external hexagon connection systems. According to the same author [3], Morse–Cone connection implants have abutment fixation by frictional locking, and there may be a microgap with irregular characteristics. However, due to the conical interface of the implant, lateral loads are resisted, preventing the loosening of the abutment. Positive or geometric locking, which prevents inclination by shielding the abutment threads from high functional loads, is the cause of this condition.

D'Ercole et al. [31] evaluated bacterial leakage of *Enterococcus faecalis* and *Aggregatibacter actinomycetemcomitans* in two different Cone–Morse connections and found no leakage in both groups. The same author [4] observed leakage over a period of 14 days in internal and external connections and reported lower rates of infiltration in internal Cone–Morse connections. Although several articles conclude that implants with internal connections perform better results under loaded and unloaded experimental conditions compared to other types of external and internal clearance-fit connections, a review by Kooutouzis et al. [18] showed that all implant connections can undergo bacterial microleakage. However, when it comes to investigations using smaller molecules, such as bacterial endotoxins, the penetration of these occurred in all specimens of implants with internal conical connections.

Despite several satisfactory studies, the results of this work show a certain bacterial infiltration in all the conometric connections analyzed. It is important to highlight that no

statistically significant differences have been found among the three types of conical connections tested in this study. Another important point is that the percentage of infiltration at the 14th day remained the same at the 90th. This could confirm that the infiltration only happens in the first days as in the study by Teixeira et al. in 2011 [3], in which the authors discovered microbial infiltration across the implant/abutment contact in periods spanning from 24 to 120 h.

As hypothesized by literature in this type of experimentation, the static outgrowth model, there is the risk of contamination during the inoculation of bacterial inside to the platform. This is a limitation of this type of study, but the repetition of several experimentation and the statistical analysis are used also to reduce this risk of bias [18].

According to Nascimento et al. [35], the stability and stress distribution of this kind of connection have reportedly been improved, and there has been reduced bacterial leakage as a result. According to reports, a more accurate adaptation of the various components and the application of appropriate torque forces are associated with superior stability of the implant abutment assembly.

Variations in the internal conical connections can be caused by differences in taper angles, lengths of the taper section of the abutment, total abutment surface areas in contact with the internal aspect of the implant, and the geometry of the anti-rotational features. [18]. Precision in engineering part manufacturing and connection type design is particularly important [36].

Khorshidi et al. [36] found that the 11° Cone–Morse connection showed less bacterial infiltration compared to a butt-joint connection. Indeed, achieving stable hard and soft tissue integration requires that the implant-abutment interface be completely sealed against microorganisms [20,37]. According to Khorshidi et al. [36], based on biological considerations, the Morse–Cone connection appears to be more effective because there is a connection between the funnel's two sides, as opposed to the butt-joint connection where the surfaces create a 90-degree angle.

Cone–Morse connections have been recommended for single implants, fixed partial prostheses, and overdenture planning due to their great mechanical stability [1,38,39]. The implant-abutment interface design and features are proposed largely to reduce crestal bone stress. The importance of implant-abutment leakage should not be overlooked in the emphasis on stress distribution [1].

Regardless of the connection type, the implants need to be manufactured with a precise seal to avoid or reduce any inflammatory responses and damage to the soft and hard tissues, which can result in bone loss surrounding the implants [1]. The precision and the manufacturing process of implants and engineering parts, and the design of the type of connection, is of paramount importance. Since the manufacturing of these parts is not efficient, they can act as reservoirs of endotoxins, by-products, and bacteria, consequently resulting in the failure of implant treatment [36,39].

Silva–Neto et al. [21,40] analyzed the effectiveness of bacterial sealing of different types of implant/abutment connections, including Morse-taper implants with solid (MT) or MT-passing screws (MTps). The latter showed a lower bacterial infiltration. Indeed, according to the same author [21], in comparison to a solid abutment, the passive screw design enables a better fit between the abutment and implant when the implant is loaded, which, over time, reduces microleakage. In addition, in our study, we found a certain lower bacterial contamination in Cone–Morse with passing screws in respect to those with solid abutment. However, no statistically significant results were found. A limitation of this study is represented by the fixation modality adopted for Group 2 of solid abutments in Cone–Morse connections. As explained in Materials and Methods, the manufacturer suggested to use a percussion with 200 gr of force by means of the use of a beaver compatible with the platform of the abutment. The expert clinician that screwed all connections tried to use the same force for all samples, but no modalities to verify the real force used during the abutment activation were available. This could have negatively

influenced the performance of Group 2, and the manufacturer should consider the production of a calibrated beaver for this type of abutment.

Due to the limited data available in the literature, the comparison of findings should be performed with caution, and other in vitro and in vivo studies should be performed on this topic in order to obtain information more adherent to clinical conditions. Another limitation of this study is that the evaluation was performed in static conditions, and that loading could interfere with the marginal gap and permit the passage of a higher quantity of bacteria, especially in Group 3, which is characterized by a higher angle of 18°, respective to the Cone–Morse connections [18]. However, despite the use of modern implant and implant connection systems, and within the limitations of the study, it is still not possible to prevent the passage of microorganisms through microgaps, even if this passage does not always result in an infection and inflammatory response of the host.

5. Conclusions

Although there is an absence of statistically significant differences, the Cone–Morse with solid abutment group showed a lower bacterial leakage in static conditions than the other groups' Cone–Morse connections. Two limitations of this study could influence these results: the static conditions of the experimentation, and the lack of a calibrated beaver for the correct activation of Cone–Morse solid abutment.

So, in further studies, it should be considered to produce a beaver able to exercise specifically 200 gr".

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