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Microbiological analysis of bacterial sealing of internal conical implants with different taper angles

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Abstract: The present study evaluated the effect of the taper angle of different internal conical connection implants and cyclic loading on the implant-abutment bacterial seal. A total of 96 implant-abutment sets were divided into eight groups. Four groups of different taper degrees with cyclic mechanical loading of 500,000 cycles per sample, with a 120-N load at 2 Hz before analysis [16DC (16-degree, cycled), 11.5DC (11.5-degree, cycled), 3DC (3- degree, cycled) and 4DC (4-degree, cycled)] were compared to four control groups without cyclic loading [16D (16-degree), 11.5D (11.5-degree), 3D (3-degree), and 4D (4-degree)]. Microbiological analysis was performed by immersing all samples in a suspension containing *Escherichia coli* and incubating them at 37°C. After 14 days, the presence of bacterial seals was evaluated. Fisher-Freeman-Halton exact tests and binomial tests were performed (5% significance level). The groups showed significant differences in bacterial seal, and mechanical load cycling improved the bacterial seal in the 3DC group. In all other groups, no significant differences in bacterial seal were found between cycled and uncycled samples. To conclude, the internal conical connection with a 3-degree taper angle showed better results than the other connection with different angles when subjected to load cycling. However, none of the angles tested were fully effective in sealing the implant-abutment interface.

Keywords: Dental Implants; Mechanical Tests; Microbiological Techniques.

Introduction

The external hexagonal connection has been associated with a higher concentration of shear stress in the peri-implant alveolar bone crest and with micromovements between the implant and the abutment that may lead to chronic contamination and to increased bone remodeling.[1-3] To improve the biomechanical performance and soft tissue healing in the peri-implant region, different prosthetic platforms and connections have emerged since the beginning of osseointegrated implant therapy. To improve implant-abutment stability and eliminate bacterial contamination of the implant, the internal conical connection was developed.[4] Observational studies on treatments with internal conical connection implants with various prosthetic rehabilitations suggest that such implant systems have the long-term predictability, high success rates, limited peri-implant bone remodeling, and low incidence of biological and prosthetic complications.[5,6]

Studies[6-8] suggest that internal conical connections can minimize bacterial infiltration, although they cannot provide complete sealing; however, when masticatory forces are applied, the contact at the implant-abutment interface tightens with compression and bacterial sealing may be improved. Studies have also shown that cyclic mechanical loading with axial forces promotes soft tissue healing around the implant-abutment connection in Morse tapers, suggesting a decrease in microgaps, thus improving the bacterial seal.[9]

The superiority of these Morse taper connections when subjected to axial loads, characterized by long-term stability, has been described in some studies.[10-12] Studies describe the internal conical connection as a safe and reliable system and as an important factor for alveolar bone crest preservation due to microgap reduction and consequent reduced risk of bacterial contamination.[12,13] The literature also reports that internal conical connections have a better shear stress distribution on the bone tissue than external hexagon connections, suggesting decreased bone remodeling and a more stable implant-abutment connection that can minimize abutment micromovement, thus improving the bacterial seal.[14,15] Some studies have also proposed that bacterial contamination through the implant-abutment interface might correlate with space size or prosthetic misfit. The degree of contamination not only varies with fit, but also depends on the degree of micromovement between components and the torque used. The incidence of oblique loads and unscrewing of the prosthetic abutment could increase infiltration.[12,16-20]

The internal geometry of the implants and the design of the prosthetic components have demonstrated a significant influence on bacterial infiltration through the implant-abutment interface, where, despite a reduced bacterial infiltration, internal conical connections were not completely effective in sealing the interface.[21-23] A previous study has compared the sealing capacity of internal conical connection implants with different internal angles (5.4, 12, 45, and 60 degrees) and suggested that all implant abutment assemblies were efficient in sealing the interface, although the smaller the internal angle, the lower the micromovements and the greater the implant-abutment surface contact, which improved sealing capacity.[24]

Therefore, accurate fit between components and mechanical stability of the prosthetic abutment are extremely important for long-term treatment success. The present study assessed the in vitro effect of the taper angle of different internal conical connection implants on implant-abutment bacterial seal subjected to cyclic mechanical loading with oblique forces. The postulated null hypothesis was that the internal angle of implant-abutment sets and cyclic loading does not influence the bacterial sealing capacity.

Methodology

A total of 96 implant-abutment sets were divided into eight groups (n = 12, with ten implant-abutment sets each group, one set used as negative control, and one as positive control). Four groups with cyclic mechanical loading of 500,000 cycles per sample, with a 120-N load at 2 Hz before analysis [16DC (16-degree, cycled), 11.5DC (11.5-degree, cycled), 3DC (3-degree, cycled) and 4DC (4-degree, cycled)] were compared to four control groups without cyclic loading [16D (16-degree), 11.5D (11.5-degree), 3D (3-degree), and 4D (4-degree)], as shown in Figure 1.

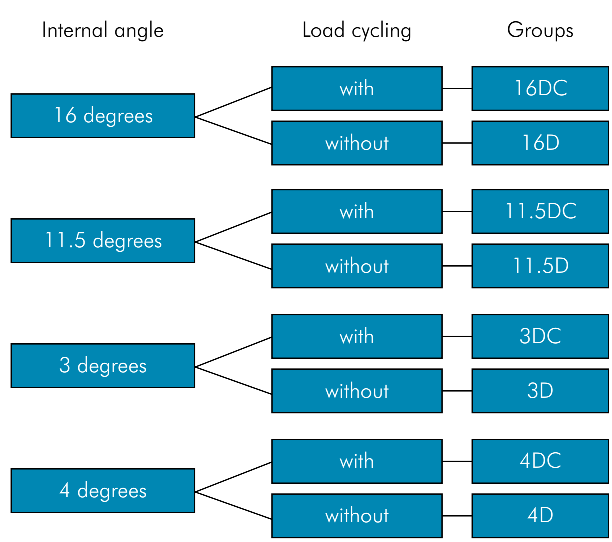

Figure 1. Division of experimental groups.

Sample preparation

A prefabricated brass pin was used to fix and stabilize the implants (SIN Implant System, São Paulo, Brazil) during the application and activation of the torque. The abutment was manually inserted into the implant, and the torque wrench recommended by the manufacturer was coupled to a manual torque gauge. A torque of 20 N cm was applied in groups 16DC, 16D, 11.5DC, 11.5D, 4DC, 4D, and a torque of 15 N cm was applied in groups 3DC and 3D, according to the manufacturer's instructions. The torque was confirmed using a digital torque meter (Torque Meter TQ-680; Lutron, Taipei, Taiwan). All samples received the activation torque at the same time, including those not subject to cyclic mechanical loading.[11]

Cyclic mechanical loading

The samples of groups 16DC, 11.5DC, and 4DC were subjected to cyclic mechanical loading using an Elquip® fatigue test machine (São Paulo, Brazil). A total of 500,000 compression loads of 120 N at a frequency of 2 Hz and an angle of 30° were used, in accordance with the recommendations of the International Organization for Standardization (ISO) standard 14801: 2007. Such a protocol is equivalent to approximately 6 months of oral function.[25]

Microbiological analysis

The experiments were conducted in a laminar flow cabinet with sterile equipment and properly trained operators. The samples were presterilized using ethylene oxide[11] and then immersed in flasks containing 75 mL of *E. coli* suspension (American Type Culture Collection 25922) at a concentration of 15×108 CFU/mL, McFarland standard 5 (Probac do Brasil, São Paulo, Brazil) and incubated for 14 days at 37°C under aerobic conditions.[11]

The culture medium was changed every 48 hours. After the incubation period, the samples were removed from each flask and dried on sterile absorbent paper to remove excess bacterial material. Each sample was rinsed three times in sterile distilled water and dried on absorbent paper to reduce contamination before the disinfection process. The implant-abutment interface was disinfected by mechanical debridement for 20 s for each sample using 0.25% peracetic acid (Proxitane Alfa, Thech Desinfecção Ltda., São Paulo, Brazil) and subsequently dried with absorbent paper.[11] All samples were then placed in a vise to separate the abutments from their implants.[11]

To confirm external decontamination, a sample was collected by rubbing a microbrush on the interface of each implant-abutment set using a 0.9% sterile saline solution before removing the abutment. Each microbrush was immersed in brain-heart infusion broth (BHI, Himedia, Mumbai, India), which was used as a control for external contamination. An extremely fine and moist microbrush (1-mm tip) was then inserted into the most apical internal part of the implant to collect bacteria that might have penetrated through the interface. Each microbrush was then immersed in a tube containing 5 mL of sterile BHI and incubated at 37°C for 48 hours. One implant-abutment set per group was used as a positive control, which was exposed to the same culture medium (*E. coli* and BHI broth); as a negative control, one implant-abutment set was simply submerged in sterile BHI broth[11] (Figure 2).

In a sample suspected of contamination, 10 μL of the culture medium was removed from the tube, plated on BHI agar and incubated at 37°C for 24 hours for macroscopic confirmation of bacterial growth. Gram staining was performed in this plate, and the slide was examined under an optical microscope (Brix, Campinas, Brazil) to confirm the presence of Gram-negative bacilli (*E. coli*) only.[11]

Statistical analysis

To assess whether there were differences in bacterial seal between the implant-abutment sets subjected or not to load cycling, Fisher-Freeman-Halton exact tests were used. To compare the effect of performing cyclic mechanical loading on the bacterial seal of each implant-abutment set, binomial tests were used. Statistical calculations were performed using the software package SPSS 23 (SPSS INC., Chicago, USA) and the website vassarstats.net, adopting a 5% significance level.

Results

A statistically significant difference was observed between the abutment/implant types in terms of

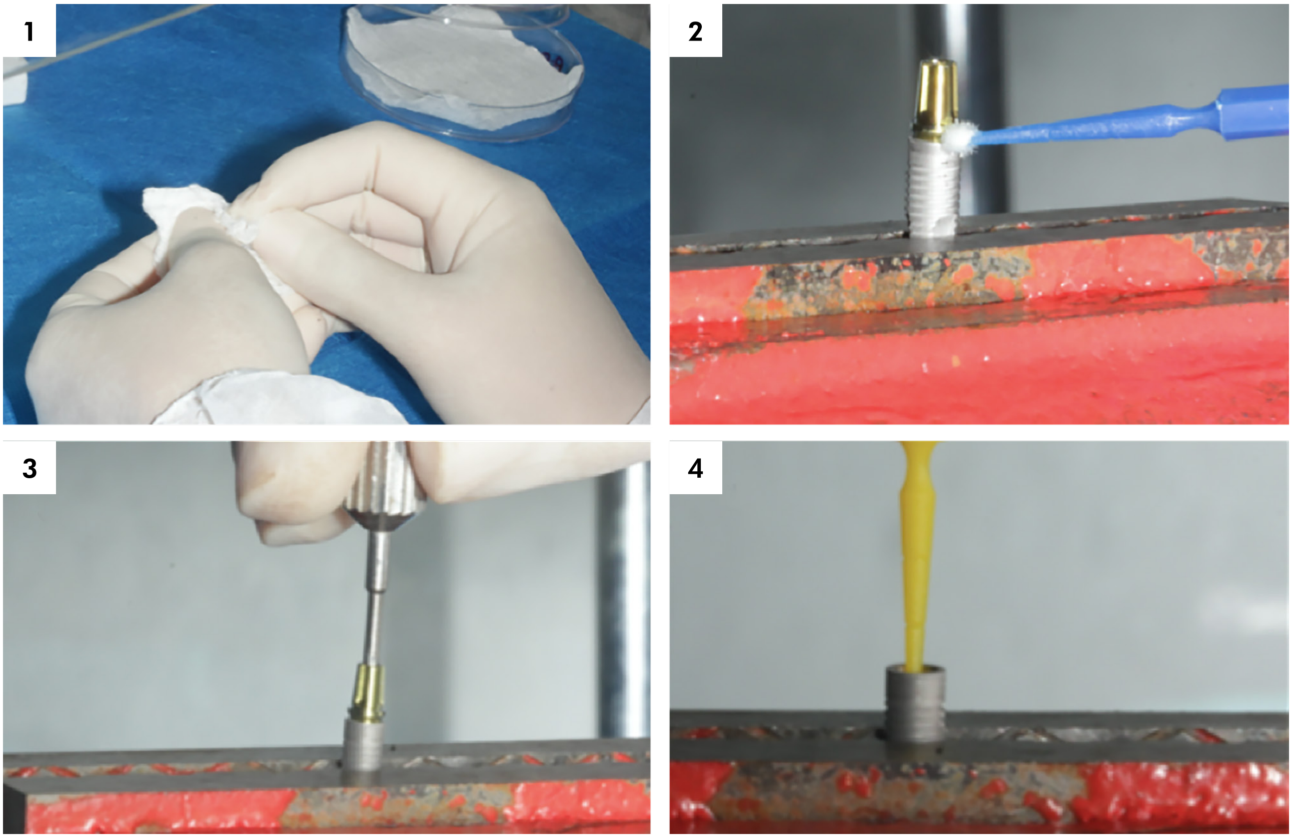

Figure 2. Representative image of bacteria colletion.1) Decontamination with 0.25% peracetic acid; 2) To confirm external decontamination, a microbrush was rubbed on the interface of the implant/abutment set using 0.9% sterile saline solution; 3) Abutment removal; 4) Bacteria collection by inserting a microbrush into the most apical internal part of the implant

microbiological sealing (Table 1). After 24 and 48 hours of mechanical load cycling, the abutment/implant with a taper angle of 3 degrees presented the lowest percentage of turbid media (20%), followed by the assemblies with taper angles of 4 (50%), 16 (90%), and 11.5 (100%) degrees.

In the absence of mechanical load cycling, however, 100% of the samples in the group with 3 degrees of internal angle had turbidity, no longer differing in microbiological sealing capacity compared to the other groups with 11.5 degrees (100%) and 16 degrees (100%). Nevertheless, the group with a taper angle of 4 degrees did not present a significant difference on the turbidity compared to the percentage after mechanical load cycling.

Regarding the effect of mechanical load cycling on the microbiological sealing of each type of abutment/implant assembly, it has been demonstrated that only the abutment/implant with a taper angle of 3 degrees presented a reduction in percent media turbidity, irrespective of incubation time. For all other abutment/implant taper angles (4, 11.5, and 16 degrees), there was no statistically significant difference in microbiological sealing between cycled and non-cycled groups (Table).

Discussion

The present study used loading cycles with oblique force, which better simulate clinical masticatory conditions. This type of loading occurs during occlusion and may induce micromovements between the prosthetic platform and the abutment, resulting in microscopic structural failure and loss of preload.[9,26,27] The literature shows that misfit between the implant and the abutment may cause mechanical and biological problems. The microgap at the interface allows bacteria to penetrate, colonizing the inside

Table. Relative frequencies (%) of turbid (T) or non-turbid (NT) media as a function of implant-abutment set and by mechanical load cycling (with or without).

Incubation time	Implant-abutment set	With load cycling		Without load cycling		p-value
		T	NT	T	NT	
24 hours	16 degrees* (16 DC and 16D)	90%	10%	100%	0%	0.305**
	11.5 degrees* (11.5DC and 11.5D)	100%	0%	100%	0%	1.000**
	3 degrees** (3DC and 3D)	20%	80%	100%	0%	< 0.001**
	4 degrees* (4DC and 4D)	50%	50%	50%	50%	1.000**
	p-value	< 0.001*		0.002*		¾
48 hours	16 degrees*** (16 DC and 16D)	90%	10%	100%	0%	0.305**
	11.5 degrees*** (11.5DC and 11.5D)	100%	0%	100%	0%	1.000**
	3 degrees*** (3DC and 3D)	20%	80%	100%	0%	< 0.001**
	4 degrees*** (4DC and 4D)	50%	50%	60%	40%	0.463**
	p-value	< 0.001*		0.009*		¾

*p-value of the Fisher-Freeman-Halton exact test applied to compare implant-abutment sets; **p-value of the binomial test applied to compare cycled and uncycled samples.

of the implant and causing circulation of bacterial endotoxins into peri-implant tissues, thus triggering a pathophysiological process that may increase bone remodeling.[7,28] *E. coli* is a facultative anaerobic, gram-negative, and mobile bacteria that measures 1.1 to 1.5 μm in diameter by 2 to 6 μm in length and is widely used for in vitro studies, particularly in contamination tests.[11,29,30] However, some studies have compared internal conical connection implants with other implants with different connection systems and shown that the former provided better bone remodeling results than the latter[5, 15] In the present study, the 16- and 11.5-degree taper groups subjected to cyclic mechanical loading with oblique loads provided worse results than the 3- and 4-degree taper groups also subjected to oblique loads, strengthening the effect of the internal angle on the fit between the internal surface of the implant and the abutment even under oblique force conditions.[31]

Significant differences in bacterial seal were found between implant-abutment sets. In the absence of cyclic mechanical loading, 100% of the 16-degree (group 16D), 11.5-degree (group 11.5D), and 3-degree taper implant-abutment sets (group 3D) showed turbidity after both 24 and 48 hours, with no significant differences between groups. However, only the 4-degree taper implant-abutment set (group 4D) showed turbidity in 50% of samples after 24 hours and in 60% of samples after 48 hours. It is possible that a taper angle closest to the true Morse taper would increase the implant-abutment set stability and improve the bacterial seal.[28] Corroborating the present study, a study comparing internal conical connection implants with different angles has shown that implants with a taper angle close to two degrees provide better results than 13-degree taper implants subjected to the torque recommended by the manufacturer.[26] The increased turbidity in group 3D, with a 3-degree taper, suggests that the torque of 15 N recommended by the manufacturer may not be sufficient to effectively seal the internal walls of the implant with the prosthetic component. Future studies are required to quantify bacteria penetration by colony-forming unit counting at the implant-abutment interface, which is a limitation of the current study. Also, correlations should be performed between taper angle, implant-abutment misfit, and bacterial sealing capacity to confirm the current results.

When subjected to cyclic mechanical loading for 24 and 48 hours, the 16-degree taper implant-abutment sets (group 16DC) showed turbidity in 100% of samples, and the 11.5-degree taper implant-abutment sets (group 11.5DC) showed turbidity in 90% of samples, with no significant differences between groups. In turn, the 4-degree taper implant-abutment set (group 4DC) showed a significantly lower proportion of turbid media (50%) than the previous sets, and an even lower turbidity occurred in 3-degree taper implant-abutment sets (group 3DC), of which only 20% failed to provide a bacterial seal. In line with the objective of the present study, horizontal and vertical masticatory forces are known to cause a deeper insertion of the abutment into the implant, thereby increasing the contact pressure.[11] In the literature, internal conical connections have shown more promising bacterial seal results when subjected to axial loads than when subjected to oblique loads. In the present study, during cyclic mechanical loading, the implant-abutment sets were subjected to 30-degree oblique loads, increasing shear stress, slipping between surfaces, and subsequent abutment wear, which may have contributed to the significant difference between groups with smaller or larger internal angles.[32]

The cyclic mechanical loading only decreased the proportion of turbid media from 100% to 20% in the 3-degree taper implant-abutment set (group 3DC) after both 24 or 48 hours of incubation. In all other groups, regardless of the incubation time, 24 or 48 hours, no significant differences in bacterial seal were found between cycled and uncycled samples. Such results further support the hypothesis of the positive effect of a small internal angle because the 4-degree implant-abutment set, despite showing no significant difference between the cycled and the uncycled groups, maintained the proportion of turbid media at 50%.

These results corroborate the literature, which suggests that the Morse taper system essentially creates a bacterial seal through a tight contact between the internal walls of the implant and the external walls of the prosthetic abutment[17]. This feature indicates that any conical metal surface connected to another at an angle smaller than 8 degrees creates a frictional fit that mechanically seals the system and that the fit decreases with the increase in angle.[31] However, the study on which the method used in this study is based compared internal conical screwless connection (Morse taper) implants with tapered screw-retained implant prosthesis interface systems under oblique loads, and none of the tested conditions effectively sealed the implant-abutment interface.[11] A possible explanation for this discrepancy is the effect of cyclic mechanical loading, which could cause a misfit of the implant-abutment set, as the internal conical connection shows better results in static methods.[26]

Given previous evidence and the results of the present study, the 3-degree taper showed better sealing results than the other taper angles when subjected to load cycling. However, all angles tested, even those closest to the true Morse taper, whether subjected to cyclic mechanical loading or not, were not fully effective in sealing the implant-abutment interface.

Conclusion

Considering the limitations of this study and based on its findings, the following conclusions are drawn:

a. Implant-abutment sets of different taper angles differed significantly in bacterial seal;
b. Cyclic mechanical loading improved the bacterial seal only in the 3-degree taper group. In all other groups, no significant difference was observed in bacterial seal between groups cycled or not with oblique forces;
c. Conical internal connections, even with taper angles close to the Morse taper, are not fully effective in sealing the implant-abutment interface.

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