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ORIGINAL ARTICLE

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Degree of bacterial microleakage at the implant-abutment junction in Cone Morse tapered implants under loaded and unloaded conditions

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ABSTRACT

Purpose: Different results have been reported on the internal colonization of Cone Morse connections under in vitro dynamic loading. The aim of the present in vitro study was to evaluate the bacterial leakage in Cone Morse implant-abutment connections, both under loaded and unloaded conditions.

Methods: A total of 20 implants with a Cone Morse taper internal connection were used in this study. Ten were loaded under a special testing equipment (Test Group), while 10 were left unloaded (Control Group). The inner part of all implants was inoculated with 0.1 µl of a viable *Enterococcus faecalis* suspension. A force of 120 N was applied to the loaded implants, for a total of 500,000 cycles at 1 Hz. All the samples were checked daily, for a total of 14 days, and presence or absence of turbidity recorded.

Results: In the unloaded assemblies, bacterial contamination was found in 2 out of 10 implant-abutment junctions, on the 12th and 13th days. In the loaded implant-abutment connections, bacterial contamination was found in 2 out of 10 implant-abutment assemblies, on the 13th and on the 14th days.

Conclusions: The resistance of the Cone Morse implant-abutment junction reported in the literature and confirmed in the present study, where no differences in the percentages of microbial leakage were found in assemblies unloaded and in those subjected to a dynamic loading procedure, could help to explain the histological results in man of a lack of peri-crestal bone resorption in Cone Morse implants, placed below the level of the alveolar crest.

Keywords: Bacterial leakage, Cone Morse connections, Dental implants, Dynamic loading, Implant-abutment junction

Introduction

The presence of an implant-abutment junction (IAJ) has been correlated with the presence of microorganisms (1). These bacteria may harbor inside the microgaps that can be found in the assemblies and they have been reported to be the cause of the inflammation of the soft tissues (abutment Infiltrated Connective Tissue or ICT) facing the IAJ, potentially producing a bone resorption of the surrounding peri-crestal bone (2-5). Bacterial migration has been correlated with tightening torque forces, micromovements of the different components during the masticatory cycles and the accuracy of the fit between implant and abutment (1). Even if the Cone Morse implant-abutment connection is considered to be the most stable and the most capable to resist penetration of microorganisms when compared with flat-to-flat or tube-in-tube connections, their possible bacterial colonization is not yet well understood (6, 7). In all implant-abutment different connections the size of the IAJ microgaps can be increased during the loading of the assemblies (2, 8, 9), introducing a so-called pumping effect (10). Higher quantities of microorganisms have been reported for loaded than unloaded external hexagon and internal hexagon implants (9). On the other hand, a minimal contamination has been reported for Cone Morse connections (2, 11). Different results have been reported on the internal colonization of Cone Morse connections under in vitro dynamic loading. do Nascimento et al (9) found no significant differences in the amount of bacteria, comparing unloaded and loaded groups, while, on the contrary, Koutouzis et al (10) found that the bacterial penetration was influenced by the loading conditions. One of 20 assemblies was found to be colonized by *Escherichia coli* in the unloaded group, while the number of colonized microgaps increased to four in the loaded group. The aim of the present in vitro study was to evaluate the bacterial leakage in Cone Morse implant-abutment connections, both under loaded and unloaded conditions.

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Methods and materials

A total of 20 implants with a Cone Morse taper internal connection (Universal II CM, Implacil, De Bortoli, Sao Paulo, Brasil) were used in this in vitro study. Ten were loaded under a special testing equipment (Test Group), while 10 were left unloaded (Control Group). To simulate cyclic loading and to mimic intraoral conditions, the Test implants were embedded in autopolymerizing acrilic resin with a custom-made stainless steel ring form. The implants were positioned using a parallelometer to ensure uniform alignment of all specimens. The implants were positioned so that the cyclic fatigue load was applied to the long axis of the implants. All embedded implants were sterilized by autoclave for 15 minutes at 121°C. After several trials, 0.1 µl was determined to be the ideal quantity of bacterial suspension for inoculation in the implant systems. *Enterococcus faecalis* (*EF*) is a Gram-positive coccus, nonmotile, facultatively anaerobic microbe, a human commensal and an important opportunistic pathogen inhabiting the gastrointestinal tracts, oral cavity and urinary tract, whose size ranges approximately 1.0-1.5 µm. This species is involved in the pathogenesis of secondary endodontic apical lesions and can also be found in root-filled teeth with no apical lesions and also in primary endodontic lesions. *EF* can survive extreme environmental conditions (acidic or basic pH, high salt concentration, the presence of heavy metals, aerobic and anaerobic). In fact, it fails to grow at 10°C and 45°C, at pH 9.6, 6.5% NaCl in broth, and it survives at 60°C for 30 min. This bacteria may invade the dentinal tubules for more than 200 microns and is able to survive without the support of other bacteria and can be resistant to a wide range of antibiotics. The inner part of ten implants was inoculated with 0.1 µl of a viable *EF* suspension with a 0.1 µl calibrated micropipette (Gilson, Middleton, WI, USA), with sterile gloves, under sterile conditions. A pure culture of *EF* (reference strain ATCC 29212) was used. For preparation of the bacterial suspension, the test organism *EF* was first plated onto MacConkey Agar without Crystal Violet (Oxoid LTD, Basingstoke, Hampshire, England) and then incubated for 24 hours at 37°C. Suspension was made from the culture by diluting a few colonies in nutrient solution (Nutrient Broth, Oxoid LTD, Basingstoke, Hampshire, England) for *EF* to a density of 0.5 McFarland Standard (1×10^8 colony forming units per mL - CFU/mL), confirmed by spectrophotometer analysis (Agilent Technologies 8453 UV, Santa Clara, USA) and subjected to a series (two series) of 10-fold dilutions in broth. As a positive control, a test tube was used with only nutrient solution and inoculated with 0.1 µl of *EF*. It showed bacterial growth with solution cloudiness, and this confirmed the viability of the microorganism throughout the experiment. As a negative control, an identified test tube was used, with only sterile nutrient solution. This was confirmed by the transparency of the solution and conventional microbial culturing techniques. Before the implants and abutments were connected, the inner part of implants was inoculated with bacterial suspension, under sterile conditions. After the implant inoculation, the abutment was carefully connected to the implant, according to the manufacturer's protocol, without touching the outer surface of the implant and using sterile gloves. An implant torque controller pre-calibrated at 30 N cm as manufacturer-recommended was used to ensure proper seating torque for all implants (Fig. 1). Subsequently, implant systems were placed in sterile cylinders. The specimens were partially immersed in Nutrient Broth (Nutrient Broth, Oxoid LTD, Basingstoke, Hampshire, England), so that only half of each element, comprising the connection implant/abutment area, was immersed in the nutrient solution and subjected to the load (Fig. 2). The whole system was maintained at a temperature of 37°C. Then, the specimens were mounted, each at a time, in a chewing simulator (Figs. 3A and B). A cyclic fatigue load (Universal Testing Machine, LLOYD LR 30K, UK) was individually applied to each specimen with a stainless steel stem through the rubber ring. A force of 120 N was applied for a total of 500,000 cycles at 1 Hz, which is within the physiologic clinical range. The samples were checked daily, for a total of 14 days, and presence or absence of turbidity recorded. Such leakage caused bacterial colonization and resulted in a cloudy solution; 1 µl of the solution was drawn with sterile 1-time-use syringes and analyzed with a Gram stain and by colony morphology in MacConkey Agar without Crystal Violet (Oxoid LTD, Basingstoke, Hampshire, England), incubated at 37°C for 24 h to confirm the purity of the microorganism which had been inoculated in the inner part of the implant and determining the presence of *EF*. A resulting growth of *EF,* confirmed that bacteria had indeed escaped from the inner part of the implant along the tested interface into the surrounding solution. The same procedure was performed with the Control implants, but without the loading.

Results

The frequencies of contamination in both tested groups were shown in Table I. In the unloaded assemblies, bacterial contamination was found in 2 out of 10 IAJ, on the 12^{th} and 13^{th} days. In the loaded implant-abutment connections, bacterial contamination was found in 2 out of 10 implant-abutment assemblies, on the 13^{th} and on the 14^{th} days. No assemblies showed contamination of the outer portions. The positive controls remained positive. The negative controls remained negative.

Discussion

A gap of 1-5 microns has been reported to be present, in retrieved human implants, between the implant and the healing screw, and this space was found to be filled by bacteria and calculus (12). Bacteria were also present inside the most apical portion of the hollow part of the implant (12). In another histological study on retrieved human implants, it has been reported that bacteria were often present between the implant and the abutment and in the internal portion of the implants (13). These results, with the finding of hollow spaces, in screw-retained abutments, between the implant and abutment components with the presence of fluids and bacteria inside the internal hollow cavities of the implants, serve to validate, in man, the results obtained from in vitro studies or animal experiments (12). Moreover, in retrieved human implants, an inflammatory infiltrate was present below the implant-abutment microgap (12), and, in an experimental study in monkeys, many inflammatory cells were found below the

Fig. 1 - Abutment connected to the implant at 30 N cm.

Fig. 2 - Implant/abutment immersed in nutrient solution, drawn by sterile syringe and analyzed by culture method.

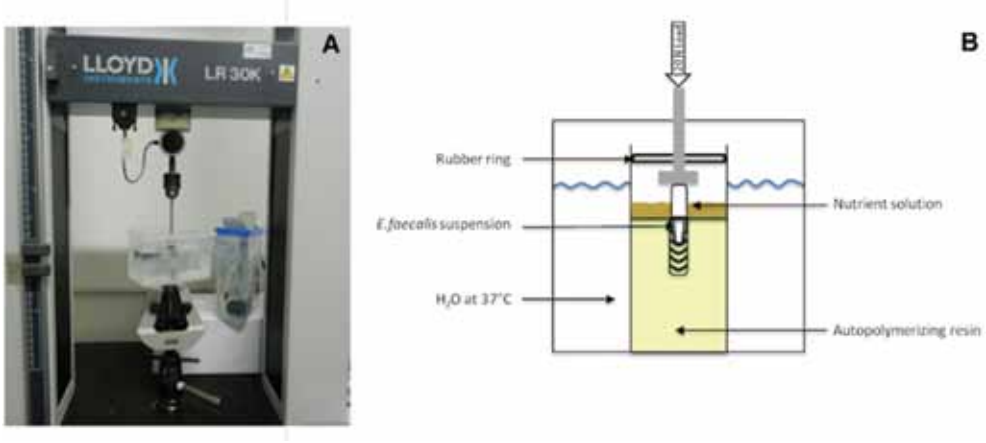

Fig. 3 - (**A**) Picture of the chewing simulator, (**B**) drawing of the chewing simulator: an implant was embedded in resin, immersed in a nutrient solution, inoculated with *E. Faecalis* suspension and subjected to cyclic fatigue load (120 N) with a stainless steel stem through the rubber ring.

TABLE I - Bacterial leakage in loaded and unloaded implants

Implants	Bacterial species	% of contamination	Days	Total
Loaded	*E. Faecalis*	2 out of 10	13th and 14th days	2 contaminated samples out of 10
Unloaded	*E. Faecalis*	2 out of 10	12th and 13th days	2 contaminated samples out of 10

implant-abutment microgap (5). There is evidence that with cyclic loading the microgap can increase in size, especially in external hexagon implants, facilitating the flow of fluids and bacteria (6, 9, 13-15). The results of the present study did not agree with the statement that overall loaded implants showed higher bacterial counts than unloaded implants (9). The present data may reflect the possible differences in the precision fit, in the degrees of taper and in the internal connection features of the implant components in the different implant systems (10, 14, 16). The reported average misfits between implant components vary greatly, from 1 to 100 microns (9). The occlusal loading of the implants produces bending forces and/or micromovements within the implant systems (9) increasing the microgap and promoting a higher fluid and bacteria infiltration (15), especially if imprecise machining of implant components or improper male-female adaptation exist (17). Cone Morse connections have been proposed to get a better stability, with reduced micromovements, of the components, providing a better bacterial seal (18). The bacterial microleakage can, probably, be reduced by obtaining an optimal component fit, a reducing or absence of micromovements, and a minimal prosthetic misfit (19-28). The length of the IAJ in Cone Morse implant-abutment assemblies could be a reason for the reported differences in the bacterial leakage (14). Implants that use mechanical frictional abutments seem to present precise adaptation in the deeper inner portions of the system, resulting in reduced micromovements (9).

Conclusion

The resistance of the Cone Morse IAJ reported in the literature and confirmed in the present study, where no differences in the percentages of microbial leakage were found in assemblies unloaded and in those subjected to a dynamic loading procedure, could help to explain the histological results in man of a lack of peri-crestal bone resorption in Cone Morse implants, placed below the level of the alveolar crest.

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References

1. Silva-Neto JP, Prudente MS, Carneiro TA, Nóbilo MA, Penatti MP, Neves FD. Micro-leakage at the implant-abutment interface with different tightening torques in vitro. J Appl Oral Sci. 2012;20(5):581-587.
2. Koutouzis T, Gadalla H, Kettler Z, Elbarasi A, Nonhoff J. The role of clohexidine on endotoxin penetration to the implant-abutment interface (IAI). Clin Implant Dent Relat Res. 2015;17(3):476-82.
3. Hermann JS, Schoolfield JD, Schenk RK, Buser D, Cochran DL. Influence of the size of the microgap on crestal bone changes around titanium implants. A histometric evaluation of unloaded non-submerged implants in the canine mandible. J Periodontol. 2001;72(10):1372-1383.
4. Persson LG, Lekholm U, Leonhardt A, Dahlén G, Lindhe J. Bacterial colonization on internal surfaces of Brånemark system implant components. Clin Oral Implants Res. 1996;7(2):90-95.
5. Piattelli A, Vrespa G, Petrone G, Iezzi G, Annibali S, Scarano A. Role of the microgap between implant and abutment: a retrospective histologic evaluation in monkeys. J Periodontol. 2003; 74(3):346-352.
6. Harder S, Podschun R, Grancicova L, Mehl C, Kern M. Analysis of the intraimplant microflora of two-piece dental implants. Clin Oral Investig. 2013;17(4):1135-1142.
7. Aloise JP, Curcio R, Laporta MZ, Rossi L, da Silva AM, Rapoport A. Microbial leakage through the implant-abutment interface of Morse taper implants in vitro. Clin Oral Implants Res. 2010;21(3):328-335.
8. Pimentel AC, Manzi MR, Sartori SG, da Graça Naclério-Homem M, Sendyk WR. In vivo effectiveness of silicone gel sheets as barriers at the inner microgap between a prosthetic abutment and an external-hexagon implant platform. Int J Oral Maxillofac Implants. 2014;29(1):121-126.
9. do Nascimento C, Miani PK, Pedrazzi V, et al. Leakage of saliva through the implant-abutment interface: in vitro evaluation of three different implant connections under unloaded and loaded conditions. Int J Oral Maxillofac Implants. 2012;27(3): 551-560
10. Koutouzis T, Mesia R, Calderon N, Wong F, Wallet S. The effect of dynamic loading on bacterial colonization of the dental implant fixture-abutment interface: an in vitro study. J Oral Implantol. 2014;40(4):432-7.
11. Larrucea Verdugo C, Jaramillo Núñez G, Acevedo Avila A, Larrucea San Martín C. Microleakage of the prosthetic abutment/implant interface with internal and external connection: in vitro study. Clin Oral Implants Res. 2014;25(9):1078-1083.
12. Orsini G, Fanali S, Scarano A, Petrone G, di Silvestro S, Piattelli A. Tissue reactions, fluids, and bacterial infiltration in implants retrieved at autopsy: a case report. Int J Oral Maxillofac Implants. 2000;15(2):283-286
13. Scarano A, Assenza B, Piattelli M, et al. A 16-year study of the microgap between 272 human titanium implants and their abutments. J Oral Implantol. 2005;31(6):269-275.
14. Steinebrunner L, Wolfart S, Bössmann K, Kern M. In vitro evaluation of bacterial leakage along the implant-abutment interface of different implant systems. Int J Oral Maxillofac Implants. 2005;20(6):875-881.
15. da Silva-Neto JP, Nóbilo MA, Penatti MP, Simamoto PC Jr, das Neves FD. Influence of methodologic aspects on the results of implant-abutment interface microleakage tests: a critical review of in vitro studies. Int J Oral Maxillofac Implants. 2012; 27(4):793-800.
16. Passos SP, Gressler May L, Faria R, Özcan M, Bottino MA. Implant-abutment gap versus microbial colonization: Clinical significance based on a literature review. J Biomed Mater Res B Appl Biomater. 2013;101(7):1321-1328.
17. Coelho AL, Suzuki M, Dibart S, DA Silva N, Coelho PG. Cross-sectional analysis of the implant-abutment interface. J Oral Rehabil. 2007;34(7):508-516.
18. Jaworski ME, Melo AC, Picheth CM, Sartori IA. Analysis of the bacterial seal at the implant-abutment interface in external-hexagon and Morse taper-connection implants: an in vitro study using a new methodology. Int J Oral Maxillofac Implants. 2012;27(5):1091-1095.
19. Al-Jadaa A1. Attin T, Peltomäki T, Schmidlin PR. Comparison of three in vitro implant leakage testing methods. Clin Oral Implants Res. 2015;26(4):e1-7.

20. Torres JH, Mechali M, Romieu O, et al. Development of a new quantitative gas permeability method for dental-implant abutment connection tightness assessment. Biomed Eng Online. 2011;10:28.
21. do Nascimento C, Miani PK, Pedrazzi V, Muller K, de Albuquerque RF Jr. Bacterial leakage along the implant-abutment interface: culture and DNA Checkerboard hybridization analyses. Clin Oral Implants Res. 2012;23(10):1168-1172.
22. Duarte ARC, Rossetti PHO, Rossetti LMN, Torres SA, Bonachela WC. In vitro sealing ability of two materials at five different implant-abutment surfaces. J Periodontol. 2006;77(11): 1828-1832.
23. Rismanchian M, Hatami M, Badrian H, Khalighinejad N, Goroohi H. Evaluation of microgap size and microbial leakage in the connection area of 4 abutments with Straumann (ITI) implant. J Oral Implantol. 2012;38(6):677-685.
24. Baggi L, Di Girolamo M, Mirisola C, Calcaterra R. Microbiological evaluation of bacterial and mycotic seal in implant systems with different implant-abutment interfaces and closing torque values. Implant Dent. 2013;22(4):344-350.
25. Assenza B, Tripodi D, Scarano A, et al. Bacterial leakage in implants with different implant-abutment connections: an in vitro study. J Periodontol. 2012;83(4):491-497.
26. D'Ercole S, Scarano A, Perrotti V, et al. Implants with internal hexagon and conical implant-abutment connections: an in vitro study of the bacterial contamination. J Oral Implantol. 2014;40(1):30-36.
27. Degidi M, Piattelli A, Shibli JA, Perrotti V, Iezzi G. Early bone formation around immediately restored implants with and without occlusal contact: a human histologic and histomorphometric evaluation. Case report. Int J Oral Maxillofac Implants. 2009;24(4):734-739.
28. Degidi M, Perrotti V, Shibli JA, Novaes AB, Piattelli A, Iezzi G. Equicrestal and subcrestal dental implants: a histologic and histomorphometric evaluation of nine retrieved human implants. J Periodontol. 2011;82(5):708-715.