An *In Vitro* Investigation Concerning the Bacterial Leakage at Implants With Internal Hexagon and Morse Taper Implant-Abutment Connections

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Purpose: *To evaluate,* in vitro, *the leakage observed in internal hexagon and Morse taper implant-abutment connections.*

Materials: *Ten specimens with internal hexagon and 10 with Cone Morse connection were used. The inner parts of 5 implants, per group, were inoculated with* Pseudomonas aeruginosa *(PS) suspension and 5 implants, per group, with* Aggregatibacter actinomycetemcomitans *(AA). The penetration of bacterial suspension into the surrounding solution was determined by observation of turbidity of the broth.*

Results: *In the internal hexagon implants, bacterial contamination was found in 2 of 5 implant-abutment assemblies seeded with the PS, and in the assemblies seeded with AA, the contamination was found in 3 samples, with a total of leaked assemblies in this group in 5 of 10. In the Cone Morse implants, bacterial contamination was found in 2 of 5 implant-abutment assemblies seeded with the PS, whereas in the assemblies seeded with AA, no contamination was found, with a total of leaked assemblies in 2 of 10.*

Conclusion: *The data confirm the high permeability to bacterial leakage of screw-retained abutment connections and the lower infiltration rates, although not significantly, of Cone Morse taper internal connections. (Implant Dent 2012;0:1–5)*

Key Words: bacterial contamination, dental implants, implant-abutment connections, microbial leakage

Leakage at the implant-abutment junction (IAJ) is a major contributing factor for periimplant inflammatory reactions.[1] Gaps and cavities between the implant and the abutment acting as a bacteriological reservoir have been described.[2] The presence of a gap between implant and abutment may cause unfavorable stress distribution on connection components, implant, and crestal periimplant bone.[3] Microbial colonization of this microgap may result in bone resorption.[2] Implant manufacturers aim to reduce the leakage by reducing the mobility of the implant-abutment connection by constructing physically tight connections with a high level of precision in a submicrometer range.[1] Very little data regarding the analysis of the microleakage at the IAJ of different implant-abutment connections are present in the literature.[1] Leakage within a short storage time (5 minutes) has been reported.[1] The degree of bacterial penetration in a specific implant system is, probably, a multifactorial condition depending on the precision of fit between the implant and abutment, the degree of micromovement between the components, and the torque forces used to connect them.[4] Transverse occlusal forces on the prosthetic restoration during function may induce bending or micromovement within the implant system, thereby increasing the gap at the component interface and inducing a pump effect between the inside of the implant and the surrounding periimplant tissue.[4] Good marginal fit of the implant components, as seen under scanning electron microscope, did not seem to be able to prevent bacterial leakage.[5] The colonization of bacteria inside the implant system and the penetration of bacteria or their products via the microgap may be a risk for soft tissue inflammation and loss of supporting bone.[6] A chemotactic stimulus

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arising at, or near, the microgap could sustain a recruitment of inflammatory cells.[7] Inflammatory cell infiltrate (infiltrated connective tissue) has been reported to occur at the IAJ.[5,6,8] A relationship between inflammatory events and bone loss seems likely.[7,9,10] It has been suggested that implants be placed with the microgap a few millimeter below the bone crest to minimize the risk of metal exposure of the top of the implant or the abutment and to allow for enough space in a vertical dimension, to develop a harmonious emergence profile.[11] However, the apical placement of the microgap has the most significant influence on the hard and soft tissues,[10] resulting in the greatest amount of bone loss.[12] The precise cause of these changes is unknown.[10] One possibility is that the microgap represents an infection site, and the host reacts with an inflammatory response.[10] Because there is still a limited body of knowledge concerning the differences in the bacterial penetration of different implant-abutment assemblies, the aim of the present *in vitro* study was an evaluation of the leakage observed in internal hexagon and Morse taper implant-abutment connections.

MATERIALS AND METHODS

A total of 20 implants were used in this *in vitro* study, 10 with a screw-retained internal hexagon abutment and 10 with a Cone Morse taper internal connection (Universal II HI and CM, respectively) (Implacil De Bortoli, Sao Paulo, Brazil).

Microbiological Examination

Ten specimens of each group were tested in the microbiological experiment. After several trials, 0.1 μL was determined to be the ideal quantity of bacterial suspension for inoculation in all implant systems. Two different bacterial sizes were used. *Pseudomonas aeruginosa* (PS) is a Gram-negative, aerobic/facultative anaerobe, rod-shaped bacterium with unipolar motility. It is considered an opportunistic human pathogen, whose size ranges from 0.5 to 1.0 μm in width and from 1.5 to 5 μm in length. *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) (AA), previously described as *Actinobacillus actinomycetemcomitans*, is a Gram-negative, facultative/anaerobic, nonmotile rod. It is an oral commensal found also in severe infections in the oral cavity, mainly the periodontium, whose size is approximately 0.4 × 1.0 μm. The inner parts of 5 implants were inoculated with 0.1 μL of a viable PS suspension and 5 implants with AA with a 0.1 μL calibrated micropipette (Gilson), with sterile gloves, under sterile conditions. A pure culture of PS (reference strain ATCC 27853) and a pure culture of AA (reference strain ATCC 29522) were used. For the preparation of the bacterial suspension, the test organism PS was first plated onto fresh cetrimide agar (Oxoid, Ltd, Hampshire, England) and incubated for 24 hours at 37°C. AA was first plated on tryptic soy agar yeast plates (Oxoid, Ltd) and then incubated for 48 hours at 37°C in 5% CO_2. Suspension was made from the culture by diluting a few colonies in nutrient broth (NB) (Oxoid, Ltd) for PS and in tryptic soy broth supplemented with yeast extract (TSBY) (Oxoid, Ltd) for AA to a density of 0.5 McFarland standard (1×10^8 colony-forming units/mL), confirmed by spectrophotometer analysis (Agilent Technologies 8453 UV, Santa Clara, CA). In all cases, 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 (Fig. 1). An implant torque controller was used to ensure proper seating torque for all implants. As a positive control, 2 identified test tubes were used with only nutrient solution and inoculated with 0.1 μL of PS and AA, respectively. They showed bacterial growth with solution cloudiness, and this confirmed the viability of the microorganisms throughout the experiment. As a negative control, 2 identified test tubes were used with only sterile nutrient solution. This was confirmed by the transparency of the solution and conventional microbial culturing techniques. Subsequent to inoculation, the assembled

AU5

Fig. 1. AU8

Fig. 2. A Cone Morse taper internal connection placed into nutrient solution.

F1

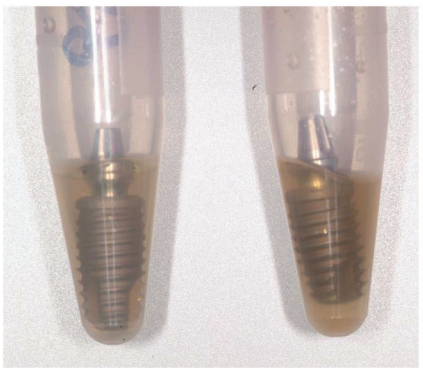

Fig. 3. Left: no contamination. Right: turbidity of the broth as a sign of bacterial penetration.

components were totally immersed for 1 minute inside the nutrient solution (NB and TSBY) in a rolling motion for the evaluation of inadvertent contamination of the external surface (Fig. 2). Tubes with a cloudy broth (indicative of colonization/contamination the outer parts of the implant) were excluded from further observation after the evaluation of bacterial growth in plates. Then, the specimens were placed into sterile Eppendorf tubes (Eppendorf, Milan, Italy), and the volume of nutrient solution required in the test vials was determined exactly for each implant system, so that the fluid level remained just above the implant-abutment interfaces. All the vials containing the assemblies, the test tubes used as external contamination control, the test tubes used as positive control, and the test tubes used as negative control were incubated at 37° C, under aerobic condition for PS and 37°C in presence of 5% CO_2 for AA. They were maintained for 28 days, and the culture broth in the vials containing the assemblies were replaced every 7 days. The possible penetration of bacterial suspension into the surrounding solution was determined by the observation of turbidity of the broth. The samples were checked daily, and the presence or absence of turbidity was recorded (Fig. 3). Such leakage caused bacterial colonization and resulted in a cloudy solution; 1 μL of the solution was analyzed with a Gram stain and by colony morphology in cetrimide agar (Oxoid, Ltd) or in tryptic soy agar yeast plates (Oxoid, Ltd), incubated at 37°C for 24 hours (48 hours for AA) to confirm the purity of the microorganism that had been inoculated in the inner part of the implant and determining the presence of PS or AA, respectively. The resulting growth of PS or AA, respectively, confirmed that bacteria had indeed escaped from the inner part of the implant along the tested interface into the surrounding solution. The experiment was not repeated because none of the test tubes showed contamination of the outer part of the implant.

Statistical Analysis

The differences between the groups were statistically analyzed using Mann-Whitney *U* test, and statistically significant differences were accepted as $P < 0.05$.

Results

In the Cone Morse implants, bacterial contamination was found in 2 of 5 implant-abutment assemblies seeded with the PS, both on the 22nd day. In the assemblies seeded with AA, no contamination was found. The total of leaked assemblies in this group was 2 of 10. In the internal hexagon implants, bacterial contamination was found in 2 of 5 implant-abutment assemblies seeded with the PS each 1 on the 16th day and on the 19th day. In the assemblies seeded with AA, the contamination was found in 3 samples on the 11th, 18th, and 20th days, respectively. The total of leaked assemblies in this group was 5 of 10. All the test tubes were examined until the 28th day because no assemblies showed contaminate the outer part of the implant. The positive controls remained positive. The negative controls remained negative (Table 1).

Statistically analysis showed no significant differences between Cone Morse and internal hexagon groups ($P = 0.2624$).

Discussion

The precise mechanism responsible for the crestal bone remodeling in 2-piece implants is not known.[13] The existence of bacterial leakage, both at the junction between the abutment and the implant, and along the abutment screw, has been reported.[13] This crestal bone resorption has not been observed around "sleeping" implants, where both exposure to microbial colonization and loading were absent.[14] It has been shown that inflammation results if the abutment loosens on the implants placed in a submerged approach, with a possible fistula formation.[15] The tightening of the abutment could eliminate the fistula in some cases.[15] In an animal experiment, after 12 months, 27% of the screws in screw-retained abutments were loosened.[16] There is a physiological reaction, with an eventual resorption at the microgap level, that may be related to the presence of bacterial contamination or micromovements of the interface.[9] The problem of a microgap between implant and abutment is biological and mechanical. The biological problem relates to the presence of bacteria that, *in vivo*, could produce a bacterial reservoir that could interfere with the long-term health of the periimplant tissues and with the long-term prognosis of the implant.[17] The mechanical problem relates to micromovement and possible loosening or

Table 1. Bacterial Leakage in Implants With Internal Hexagon and Morse Taper Implant-Abutment Connections Inoculated With *Pseudomonas aeruginosa* and *Aggregatibacter actinomycetemcomitans*

Implants	Bacterial Species	Number of Contamination	Days	Total
Cone Morse	*P. aeruginosa*	2 out of 5	Both on the 22nd day	2 contaminated samples out of 10
	A. actinomycetemcomitans	0 out of 5		
Internal hexagon	*P. aeruginosa*	2 out of 5	16th day 19th day	5 contaminated samples out of 10
	A. actinomycetemcomitans	3 out of 5	11th day 18th day 19th day	

fracturing of screw-retained abutments.[17] In a histological study of 2 human screw-retained implants, retrieved at autopsy, a gap was present between the implant and the healing screw, and this space was filled by bacteria and calculus.[18] Bacteria were also present in the most apical portions of the hollow part of the implants, and an inflammatory infiltrate was present in the connective periimplant tissues.[18] The presence of the inflammatory infiltrate (infiltrated connective tissue) confirms, in an *in vivo* human study, the data reported in animal experimental studies. In a retrospective microscopic study of human implants with a screwed-retained abutment retrieved after a long period (up to 16 years) of clinical service, bacteria were often found in the microgaps between implant and abutment and in the internal portion of the implants.[17] In a retrospective histological study in monkey, it was found that no inflammatory infiltrate was present when the implants had been inserted with the microgap above the alveolar crest level. On the contrary, many inflammatory cells were present in the area of the IAJ and inside the gap, with many osteoclasts resorbing bone, in implants that had been placed at the level or below the alveolar crest.[8] Implant-abutment interface may affect the potential risk for invasion of oral microorganisms into the fixture-abutment interface microgap under dynamic-loading conditions.[18] Penetration of bacteria may then occur, *in vivo*, from an external source to the inner portion of the implant.[19] The use of bacteria, such as PS and AA, seems relevant for *in vitro* studies because these microorganisms have been found frequently in periimplantitis lesions.[20,21] Moreover, the 2 bacteria used were different and divided into small (AA) and medium-to-large (PS) sizes, nonmotile rod (AA) and with unipolar motility (PS), facultative/anaerobic (AA) and aerobic/facultative anaerobe (PS), to try to repeat *in vitro* all the oral cavity *in vivo* conditions. However, no differences were observed between the 2 bacteria used, during the bacterial leakage along the interface of the 2 different tested implants and abutments.

The Cone Morse taper internal connection, idealized to be completely stable with absence of micromovements between the parts during function, seems to be able to resist more to the bacteria penetration due to of their self-locking characteristics.[22] In an *in vitro* analysis on strain distributions by varying the fixture-abutment design and fixture alignment, the authors concluded that the internal hexagon and Morse taper joints did not reduce the microstrain around implants.[23] Although for both internal connection and external hexed systems, loss of screw tightness can be correlated with plastic deformation of the screw, this does not seem to be true, however, for a conical interface implant system.[24] A recent study confirmed the very low permeability to bacteria of the conical implant-abutment connection, and the high prevalence of bacterial penetration of screw-retained implant-abutment assemblies.[25] Therefore, connection rigidity does not necessarily involve bacterial sealing, which in some ways explains the good clinical performance of tapered connection implants with respect to the maintenance of bone levels, even when the interface is placed below the bone crest.[26]

CONCLUSION

Finally, the results of the present *in vitro* study show that bacterial contamination occurs in different types of implant-abutment connections, even if with different percentages. Furthermore, it must be pointed out that in conical implant-abutment connection, the bacterial contamination occurred quite lately during the course of the experiment (on the 22nd day), whereas the contamination was always earlier in the butt-joint connection implants. It must also be pointed out that according to the knowledge of the authors, this is the only study present in the literature with a longer observation period (28 days), while most of the previous researches used only a study period of 7 or 14 days. This fact must be borne in mind and could be relevant in designing future studies.

DISCLOSURE

The authors claim to have no financial interest, either directly or indirectly, in the products or information listed in the article.

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