Implants With Internal Hexagon and Conical Implant-Abutment Connections: An In Vitro Study of the Bacterial Contamination

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Prevention of microbial leakage at the implant-abutment junction is a major challenge for the construction of 2-stage implants in order to minimize inflammatory reactions and to maximize bone stability at the implant neck. The aim of the present in vitro study was an evaluation of the leakage observed over a period of 28 days in Cone Morse taper internal connections and in screwed-abutments connections. In the present study 10 specimens of Cone Morse (Group 1) and 10 of internal hexagon (Group 2) implants 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 possible penetration of bacterial suspension into the surrounding solution was determined by the observation of turbidity of the broth. In Group 1, bacterial contamination was found in 3 out of 5 implant-abutment assemblies seeded with the PS and in 2 samples out of 5 in the assemblies seeded with AA, with a total of leaked assemblies in this group of 5 out of 10. In Group 2, bacterial contamination was found in 4 out of 5 implant-abutment assemblies seeded with the PS, and in 4 out of 5 samples seeded with AA, with a total of leaked assemblies of 8 out of 10. The present data confirm the reported high permeability to bacterial leakage of screw-retained abutment connections, and the lower infiltration rates—*although not significantly*—of Cone Morse taper internal connections.

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

INTRODUCTION

Bacterial contamination of implants can occur.[1] Bacterial leakage through the implant-abutment junction (IAJ) has already been reported.[1] Prevention of microbial leakage at the IAJ is a major challenge for the construction of 2-stage implants in order to minimize inflammatory reactions and to maximize bone stability at the implant neck.[2] Several investigators tried to quantify microbial leakage of dental implants,[2] but the literature concerning the quantification of microleakage and fluid passage between different connection designs is sparse.[3] This leakage could determine an inflammatory process in the peri-implant tissues near the level of the alveolar bone crest.[4,5] Broggini et al[6] demonstrated an increase in inflammatory cells in the peri-implant soft tissues at the level or slightly coronal to the IAJ. Fistulae in the peri-implant soft tissues have been frequently reported.[4] Even Cone Morse taper im-

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plants seem to be unable to completely prevent bacterial leakage.[4] In an in vitro study, comparing bacterial infiltration in 2 implants with different Morse taper conical connections (Bicon Systems, Boston, Mass; ANKYLOS, Dentsply Implants Manufacturing GmbH, Mannheim, Germany), it was found that 20% of the implants showed evidence of bacterial leakage, following incubation, all in the second day.[4] Jansen et al[7] found after 14 days of incubation, contamination in 50% of the ANKYLOS and in 69% of the Astra implants. Location of the microgap near the alveolar crest could also be responsible for more than 1 mm of bone loss, reported in the first year of implant loading.[8] The microgap has been found to have a significant influence on crestal bone levels around 2-piece implants.[9] Two-piece implants showed moderate to severe degrees of inflammation, while minimal signs of inflammation were found in 1-piece implants, and, furthermore, a more apical location of the microbial infiltrate could produce a more anaerobic bacterial infiltrate with a consequent more severe degree of inflammation.[9]

Since 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, over a period of 28 days, in Cone Morse taper internal connections and in screwed-abutments connections.

Materials and Methods

In the present study, Cone Morse implants 4.25 × 15, with a universal 3.30 × 4 cm abutment, and internal hexagon (HI) implants 6 × 15, with a fixed-air HI 5.00 × 2.00 abutment (Dentoflex, São Paulo, Brazil) were used. 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.

Microbiological examination

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 wide and from 1.5 to 5 μm long. *Aggregatibacter 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 Italia SRL, Cinisello B [MI], Italy), 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 preparation of the bacterial suspension, the test organism PS was first plated onto fresh cetrimide agar (Oxoid LTD, Basingstoke, Hampshire, UK) and incubated for 24 hours at 37°C. AA was first plated on tryptic soy agar yeast plates (Oxoid) and 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) for PS and in tryptic soy broth supplemented with yeast extract (TSBY; Oxoid) for AA 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, CA, USA). 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. An implant torque controller pre-calibrated at 35 N cm as manufacturer-recommended 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 components were totally immersed for 1 min inside the nutrient solution (NB and TSBY) in a rolling motion for evaluation of inadvertent contamination of the external surface. Tubes with

TABLE

Bacterial leaking in implants inoculated with *Pseudomonas aeruginosa* and *Aggregatibacter actinomycetemcomitans**

Implants	Bacterial species	% of contamination	Days	Total
Cone Morse	PS	3 out of 5	All on the 22nd day	5 contaminated samples out of 10
	AA	2 out of 5	22nd day 24th day	
Internal hexagon	PS	4 out of 5	1 on the 13th day 3 on the 21st day	8 contaminated samples out of 10
	AA	4 out of 5	8th day 16th day 21st day 24th day	

*PS indicates *Pseudomonas aeruginosa*; AA, *Aggregatibacter actinomycetemcomitans*.

a cloudy broth (indicative of colonization/contamination the outer parts of the implant) were excluded from further observation after 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 IAJ. 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 was 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 presence or absence of turbidity recorded. Such leakage caused bacterial colonization and resulted in a cloudy solution, 1 μL of the solution was analysed with a Gram stain and by colony morphology in cetrimide agar (Oxoid) or in tryptic soy agar yeast plates (Oxoid), incubated at 37°C for 24 h (48 h for AA) to confirm the purity of the microorganism, which had been inoculated in the inner part of the implant and determining the presence of PS or AA, respectively. A 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 Test; statistically-significant differences were accepted as $P < .05$.

RESULTS

In the Cone Morse implants, bacterial contamination was found in 3 out of 5 implant-abutment assemblies seeded with the PS, all on the 22nd day. In the assemblies seeded with AA, the contamination was found in 2 samples out of 5, respectively on the 22nd and 24th days. The total of leaked assemblies in this group was 5 out of 10 assemblies. In the internal hexagon implants, bacterial contamination was found in 4 out of 5 implant-abutment assemblies seeded with the PS, respectively 1 assembly on the 13th day, and 3 assemblies on the 21st day. In the assemblies seeded with AA, the contamination was found in all 4 samples, respectively on the 8th, 16th, 21st, and 24th days. The total of leaked assemblies in this group was 8 out of 10 (Table). All the test tubes were examined until the 28th day because none of the assemblies showed contamination of the outer part of the implant. The positive control remained positive. The negative control remained negative.

Statistically analysis showed no significant differences between Cone Morse and internal hexagon groups ($P = .2595$).

DISCUSSION

Explanations for the presence of the microgap include imprecise machining of implant parts,

Figures 1–4. Figure 1. The inner part of implants inoculation with a viable bacteria suspension. **Figure 2.** An assembled implant-abutment with an implant torque controller. **Figure 3.** Cone Morse implants placed into nutrient solution. Left: no contamination. Right: turbidity of the broth as sign of bacterial penetration. **Figure 4.** The internal hexagon implants placed into nutrient solution. Left: no contamination. Right: turbidity of the broth as sign of bacterial penetration.

excessive torque during abutment installation leading to part distortion, and improper male-female adaptation.[3] Microbial pathways are created along the interfaces toward internal implant and superstructure components and vice versa.[10] This phenomenon is considered the most plausible explanation for the development of an inflammatory cell infiltrate along the interfaces.[10] The presence of voids certainly facilitates the bacterial migration and the presence of bacteria inside the implant, which could be the result of either contamination during the first or second stage of

implant placement or transmission of bacteria from the oral environment after prosthesis placement.[11] Screw loosening is a complication that can damage interfaces in implant components, favoring contamination of its internal parts by microorganisms.[12] Higher bacterial counts were found in the groups of implants where the screws had been tightened, loosened, and retightened.[12] This reservoir of bacteria could, possibly, facilitate the development of peri-implant inflammation.[6] It is unknown if different implant-abutment connections would yield a different distribution or intensity of inflammatory cell recruitment as compared with the flat, butt-joint interface.[6] The present data confirm the lower infiltration rates of Cone Morse taper internal connections,[4,13–15] and the reported high permeability to bacterial leakage of screw-retained abutment connections.[1,3,7,13]

The use of bacteria, such as PS and AA, seems relevant for in vitro studies in the context that these microorganisms have been found frequently in peri-implantitis lesions.[16,17] Moreover, 2 bacteria used were different and divided into small (AA) and medium-large sizes (PS), nonmotile rod (AA) and with unipolar motility (PS), facultative/anaerobic (AA), and aerobic/facultative anaerobe (PS), to repeat in vitro all the oral cavity in vivo conditions. No difference between the 2 used bacteria was observed during the bacterial leakage along the interface of the tested implants and their abutments.

In this study a longer observation period was used (28 days vs the 5, 7, or 14 days reported in other studies); this fact could be important, because most of the samples that showed microbial leakage did so after 14 days. This fact should be probably kept in mind when designing future studies. In conclusion, in screw-retained abutments, the microgap can be a critical factor for bacterial contamination, while the passage of bacteria was lower—although not significantly—in Cone Morse connections.

Abbreviations

AA: *Aggregatibacter actinomycetemcomitans*
IAJ: implant abutment junction
NB: nutrient broth
HI: internal hexagon
PS: *Pseudomonas aeruginosa*
TSBY: tryptic soy broth supplemented with yeast extract

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