Riference OP-BI1402001P1

Publication date

Febbraio 2014

Event

Publication
Implant Dentistry 2014;
vol. 23, Number 2

Headline scientific research

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Bacterial Leakage in Morse Cone Internal Connection Implants Using Different Torque Values: An *In Vitro* Study

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Purpose: *The aim of this study was to assess whether there was a decrease of bacterial leakage with increasing torque values in conical Morse Cone connection implants.*

Methods: *A total of 30 Morse Cone conical tapered implants (10 implants per group) were used in this study. The abutments were connected to the implants with 20 N (group 1), 30 N (group 2), and 40 N (group 3) insertion torque values. The inner parts of 5 implants, per group, were inoculated with* Pseudomonas aeruginosa *suspension and the remaining 5 implants, per group, with* Aggregatibacter actinomycetemcomitans. *The penetration of bacteria into the surrounding solution was determined by the observation of turbidity of the broth.*

Results: *In groups 1 and 2, bacterial contamination was found in 2 of the 10 implants, only in the specimens seeded with* P. aeruginosa. *In group 3, no contaminated samples were found.*

Conclusion: *This study demonstrated that with increased insertion torque values in Morse Cone connection, the bacterial leakage is reduced. (Implant Dentistry 2014;23:1–5)*

Key Words: bacterial leakage, Morse Cone implants, in vitro studies, torque forces

Soft tissue fistulae were frequently observed clinically in Brånemarkimplants.[1] One of the most important problems related to 2-stage implants is the decrease or the complete elimination of bacterial leakage at the level of the implant-abutment junction (IAJ) to reduce inflammatory processes at the interface and to maintain the long-term stability and level of the periimplant alveolar crest.[2] Several *in vitro* studies have demonstrated the occurrence of bacterial leakage at the level of the implant-abutment microgap with possible colonization of the gaps and cavities present inside the implant and between implant and abutment.[2–22] These cavities may act as a bacterial reservoir that could interfere with periimplant tissue health.[1,15] These bacteria or their products have been hypothesized to be correlated to the chronic inflammatory infiltrate described at the level of the IAJ[23–26] and to the crestal bone resorption observed especially after the first year of function.[27–33]

The degree of bacterial leakage in the different implant systems probably are related to several factors, that is, precision of the fit between implants and abutments, micromovements between implants and abutments, and torque values used for the connection of the abutments to the implant body.[5,19,34] The construction of more precise and physically tight connections may be important in the reduction of the bacterial leakage.[2] Morse conical taper connections have been shown to have a reduced bacterial leakage.[9–11,19–22] Bacterial leakage was found to be significantly less as the torque values increased.[12] The sets that did not receive the recommended torque values (positive controls) showed turbidity.[11]

The aim of this study was to assess whether there was a decrease of bacterial leakage with increasing torque values in conical Morse Cone connection implants.

Materials and Methods

A total of 30 Morse Cone tapered implants (Biological Conical Connection Oralplant; Oralplant, Cordenons, PD, Italy) (Fig. 1, A and B) were used in this *in vitro* study; 10 specimens of each group were tested in the microbiological experiment. They were inoculated with 2 different bacterial suspensions (*Pseudomonas aeruginosa* and *Aggregatibacter actinomycetemcomitans*) (Fig. 2), and then the abutments were connected to the implants with 20 N (group 1), 30 N (group 2), and 40 N (group 3) insertion torque values (Fig. 3).

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ISSN 1056-6163/14/02302-001
Implant Dentistry
Volume 23 • Number 2

DOI: 10.1097/ID.0000000000000044

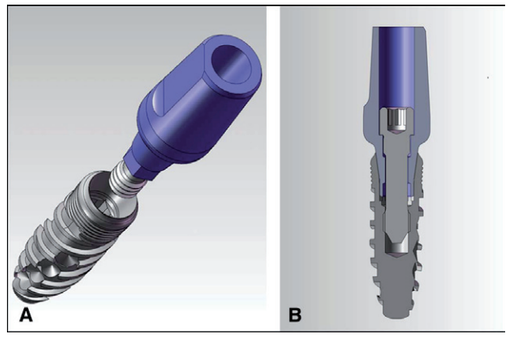

Fig. 1. A, 3D reconstruction of the Morse Cone conical tapered implant. **B**, Longitudinal section showing the components of the implant-abutment connection.

Microbiological Examination

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* 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* (*A. actinomycetemcomitans*), 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 part of 5 implants, for group, were inoculated with 0.1 μL of a viable *P. aeruginosa* suspension and 5 implants with *A. actinomycetemcomitans* with a 0.1 μL calibrated micropipette (Gilson), with sterile gloves, under sterile conditions. A pure culture of *P. aeruginosa* (reference strain ATCC 27853) and a pure culture of *A. actinomycetemcomitans* (reference strain ATCC 29522) were used. For preparation of the bacterial suspension, the test organism *P. aeruginosa* was first plated onto fresh cetrimide agar (Oxoid Ltd., Basingstoke, Hampshire, England) and then incubated for 24 hours at 37°C. *Aggregatibacter actinomycetemcomitans* 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 *P. aeruginosa* and in tryptic soy broth supplemented with yeast extract (TSBY) (Oxoid Ltd.) for *A. actinomycetemcomitans* to a density of 0.5 McFarland Standard (1×10^8 colony forming units per milliliter—CFU/mL), confirmed by spectrophotometer analysis (Agilent Technologies 8453 UV, Santa Clara, CA) and subjected to a series (2 series) of 10-fold dilutions in broth. 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. As a positive control, 2 identified test tubes were used with only nutrient solution and inoculated with 0.1 μL of *P. aeruginosa* and *A. actinomycetemcomitans*, 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 minute inside the nutrient solution (NB and TSBY) in a rolling motion for the evaluation of inadvertent contamination of the external surface. Tubes with a cloudy broth (indicative of colonization/contamination the outer parts of the implant) were excluded from further observations 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

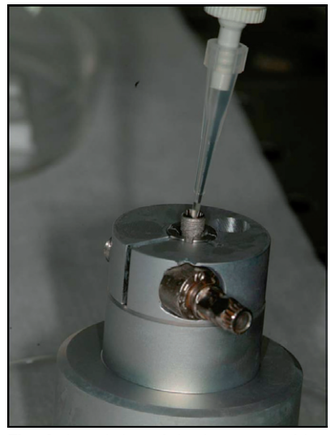

Fig. 2. Inoculation of the inner part of implants with a viable bacteria suspension.

Fig. 3. An implant-abutment connection assembled with an implant torque controller.

Table 1. Bacterial Leakage in Implants Assessed at Varying Closing Torques Inoculated With *P. aeruginosa* and *A. actinomycetemcomitans*

Implants	Bacterial Species	Percentage of Contamination	Days	Total
Group 1, 20 N	*P. aeruginosa*	2 of the 5	Both on the 6th day	2 contaminated samples of the 10
	A. actinomycetemcomitans	0 of the 5		
Group 2, 30 N	*P. aeruginosa*	2 of the 5	Both on the 13th day	2 contaminated sample of the 10
	A. actinomycetemcomitans	0 of the 5		
Group 3, 40 N	*P. aeruginosa*	0 of the 5		No contaminated sample of the 10
	A. actinomycetemcomitans	0 of the 5		

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 *P. aeruginosa* and 37°C in the presence of 5% CO_2 for *A. actinomycetemcomitans.* They were maintained for 14 days, and the culture broth in the vials containing the assemblies were replaced every 4 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. 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 *A. actinomycetemcomitans*) to confirm the purity of the microorganism that had been inoculated in the inner part of the implant and to determine the presence of *P. aeruginosa* or *A. actinomycetemcomitans,* respectively. A resulting growth of *P. aeruginosa* or *A. actinomycetemcomitans*, 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.

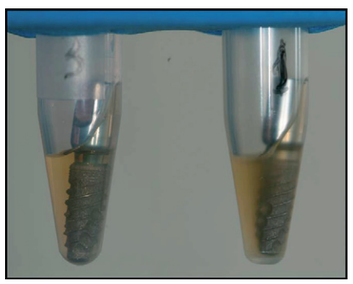

Fig. 4. Abutments connected to the implants with 20 N (group 1) insertion torque values and placed into nutrient solution. Left: no contamination. Right: turbidity of the broth as sign of *P. aeruginosa* penetration.

RESULTS

The number of assemblies showing bacterial growth in the nutrient solution monitored over 14 days is shown in Table 1.

In the group 1 (20 N), bacterial contamination was found in 2 of the 5 implant-abutment assemblies seeded with the *P. aeruginosa*, all on the sixth day (Fig. 4). Two assemblies at 30 N and inoculated with *P. aeruginosa* showed the evidence of bacterial leakage after 13 days of incubation. In all 4 positive cases, the *P. aeruginosa* reference strain was identified as the reason for the solution's cloudiness, plated onto fresh cetrimide agar, and the colonies were identified positive to colonizing bacterium. In the assemblies at 20 N and 30 N seeded with *A. actinomycetemcomitans*, no contamination was found. In the group 3 (40 N), no contamination was found either in the specimens seeded with *P. aeruginosa* or *A. actinomycetemcomitans*.

All the test tubes were examined until the 14th day because no assemblies showed contamination of the outer part of the implant. The positive control showed cloudy broths, which confirmed the viability of the microorganisms throughout the experiment. The negative control, used to check for microbial crosscontamination during the experiment, had clear broths.

DISCUSSION

When abutments are connected to implants, microgaps and cavities are produced at the interface and in the inner portions of the implants. These gaps and cavities have been shown to be able to be colonized by bacteria. Bacteria and their products can have a negative impact at the level of the periimplant soft tissues and can, also, be involved in the resorption of the periimplant crestal bone, observed especially in the first year of function.[26–31] Implant manufacturers have tried to decrease or eliminate the occurrence of this bacterial leakage and its negative influence on the periimplant tissues by proposing more tight and stable connections, with a more precision fit between the implant-abutment components, by moving the microgap in an inward direction (platform switching), by using different torque values.[34] In previous studies from our laboratory, it was found a lesser amount of bacterial microleakage with the use of Morse Cone internal tapered connections.[20–22] These results have been confirmed in the literature.[2,9,11,14,19] This type of connection has been reported to have a superior stability and distribution of the stress[35] and to show a lesser quantity of bacterial leakage.[9] A better stability of the implant-abutment assembly has been reported to be related to a more precise adaptation of the different components and to the use of suitable torque forces.[5,18,34]

Not many investigations about the relationship between increasing torque values and bacterial leakage have been reported in the literature. In a recent systematic review of the literature, da Silva-Neto et al[34] reported that implants that had received higher torque forces to connect the abutments to the implants showed the presence of lesser amounts of leakage. Gross et al[12] found that a significant decrease of microleakage was found for increasing torque values, and the author's assumption was that this decrease was related to a more precise fit between the assembly components reported with higher torque values. Jaworski et al[11] have shown that the implant-abutment assemblies where the recommended values were not used, presented a turbidity of the medium. However, Coelho et al[17] have reported that the use of too high torque forces could produce a distortion of some portions of the interface with an increase of the microgap. The results of the present *in vitro* study supported the data reported in the literature of a decrease of leakage with increasing torque forces.

In conclusion, this study demonstrated that increasing insertion torque values in Morse Cone connection reduced the leakage because no bacteria were found when abutments were connected to implants with a 40 N insertion torque.

DISCLOSURES

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

ACKNOWLEDGMENTS

This work was partially supported by the Ministry of Education, University and Research (MIUR), Rome, Italy.

REFERENCES

1. Aloise JP, Curcio R, Laporta MZ, et al. Microbial leakage through the implant-abutment interface of Morse taper implants in vitro. *Clin Oral Implants Res.* 2010;21:328–335.

2. Harder S, Dimaczek B, Açil Y, et al. Molecular leakage at implant abutment-connection—In vitro investigation of tightness of internal conical implant-abutment connections against endotoxin penetration. *Clin Oral Investig.* 2010;14:427–432.

3. Jansen VK, Conrads G, Richter EJ. Microbial leakage and marginal fit of the implant-abutment interface. *Int J Oral Maxillofac Implants.* 1997;12:527–540.

4. Persson LG, Lekholm U, Leonhardt A, et al. Bacterial colonization on internal surfaces of Brånemark system implant components. *Clin Oral Implants Res.* 1996;7:90–95.

5. Steinebrunner L, Wolfart S, Bössmann K, et al. In vitro evaluation of bacterial leakage along the implant-abutment interface of different implant systems. *Int J Oral Maxillofac Implants.* 2005;20:875–881.

6. Quirynen M, van Steenberghe D. Bacterial colonization of the internal part of two-stage implants. An in vivo study. *Clin Oral Implants Res.* 1993;4:158–161.

7. Scarano A, Assenza B, Piattelli M, et al. Retrospective evaluation of the microgap between implants and abutments in 272 titanium implants retrieved from man: A 16 years experience (1989-2004). *J Oral Implantol.* 2005;31:269–275.

8. Orsini G, Fanali S, Scarano A, et al. Tissue reactions, fluids and bacterial infiltration in implants retrieved at autopsy: A case report. *Int J Oral Maxillofac Implants.* 2000;15:283–286.

9. Fauroux MA, Lavallois B, Yachouh J, et al. Assessment of leakage at the implant-abutment connection using a new gas flow method. *Int J Oral Maxillofac Implants.* 2012;27:1409–1412.

10. 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;27:551–560.

11. Jaworski ME, Melo AC, Piche CM, et al. 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;27:1091–1095.

12. Gross M, Abramovich I, Weiss EI. Microleakage at the abutment-implant interface of osseointegrated implants: A comparative study. *Int J Oral Maxillofac Implants.* 1999;14:94–100.

13. Dias EC, Bisognin ED, Harari ND, et al. Evaluation of implant-abutment microgap and bacterial leakage in five external-hex implant systems: An in vitro study. *Int J Oral Maxillofac Implants.* 2012;27:346–351.

14. do Nascimento C, Miani PK, Watanabe E, et al. In vitro evaluation of bacterial leakage along the implant-abutment interface of an external hex-implant after saliva incubation. *Int J Oral Maxillofac Implants.* 2011;26:782–787.

15. Dibart S, Warbington M, Su MF, et al. In vitro evaluation of the implant-abutment bacterial seal: The locking taper system. *Int J Oral Maxillofac Implants.* 2005;20:732–737.

16. do Nascimento C, Barbosa RES, Issa JPM, et al. Bacterial leakage along the implant-abutment interface of premachined or cast components. *Int J Oral Maxillofac Surg.* 2008;37:177–180.

17. Coelho PG, Sudack P, Suzuki M, et al. In vitro evaluation of the implant abutment connection sealing capability of different implant systems. *J Oral Rehabil.* 2008;35:917–924.

18. do Nascimento C, Pedrazzi V, Miani PK, et al. Influence of repeated screw tightening on bacterial leakage along the implant-abutment interface. *Clin Oral Implants Res.* 2009;20:1394–1397.

19. Tesmer M, Wallet S, Koutouzis T, et al. Bacterial colonization of the dental implant fixture-abutment interface: An in vitro study. *J Periodontol.* 2009;80:1991–1997.

20. Assenza B, Tripodi D, Scarano A, et al. Bacterial leakage in implants with different implant-abutment connections: An in vitro study. *J Periodontol.* 2012;83:491–497.

21. D'Ercole S, Scarano A, Perrotti V, et al. Implants with external hexagon and conical implant-abutment connections: An in vitro study of the bacterial contamination. *J Oral Implantol.* 2011.

22. Tripodi D, Vantaggiato G, Scarano A, et al. An in vitro investigation concerning the bacterial leakage in implants with internal hexagon and Morse taper implant-abutment connections. *Implant Dent.* 2012;21:335–339.

23. Abrahamsson I, Berglundh T, Wennström J, et al. The peri-implant hard and soft tissues at different implant systems. A comparative study in the dog. *Clin Oral Implants Res.* 1996;7:212–219.

24. Abrahamsson I, Berglundh T, Glantz PO, et al. The mucosal attachment at different abutments. An experimental study in dogs. *J Clin Periodontol.* 1998;25:721–727.

25. Abrahamsson I, Berglundh T, Lindhe J. Soft tissue response to plaque formation at different implant systems. A comparative study in the dog. *Clin Oral Implants Res.* 1998;9:73–79.

26. Piattelli A, Vrespa G, Petrone G, et al. Role of the microgap between

implant and abutment: A retrospective histologic evaluation in monkeys. *J Periodontol.* 2003;74:346–352.

27. Hermann JS, Buser D, Schenk RK, et al. Biological width around one and two-piece titanium implants. *Clin Oral Implants Res.* 2001;12:559–571.

28. Hermann JS, Schoolfield JD, Schenk RK, et al. Influence 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;72:1372–1383.

29. Hermann JS, Buser D, Schenk RK, et al. Crestal bone changes around titanium implants. A histometric evaluation of unloaded non-submerged and submerged implants in the canine mandible. *J Periodontol.* 2000;71:1412–1424.

30. Assenza B, Scarano A, Petrone G, et al. Crestal bone remodeling in loaded and unloaded implants and the microgap: A histologic study. *Implant Dent.* 2003;12:235–241.

31. Hermann JS, Cochran DL, Nummikoski PV, et al. Crestal bone changes around titanium implants. A radiographic evaluation of unloaded non-submerged and submerged implants in the canine mandible. *J Periodontol.* 1997;68:1117–1130.

32. Donovan R, Fetner A, Koutouzis T, et al. Crestal bone changes around implants with reduced abutment diameter placed non-submerged and at subcrestal positions: A 1-year radiographic evaluation. *J Periodontol.* 2010;81:428–434.

33. Weng D, Nagata MJH, Bell M, et al. Influence of microgap location and configuration on peri-implant bone morphology in nonsubmerged implants: An experimental study in dogs. *Int J Oral Maxillofac Implants.* 2010;25:540–547.

34. da Silva-Neto JP, Nobilo MA, Penatti MPA, et al. Influence of methodologic aspects on the results on implant-abutment interface microleakage tests: A critical review of in vitro studies. *Int J Oral Maxillofac Implants.* 2012;27:793–800.

35. Seetoh YL, Tan KB, Chua EK, et al. Load fatigue performance of conical implant-abutment connections. *Int J Oral Maxillofac Implants.* 2011;26:797–806.

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