Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/locate/fdj

Bacterial leakage of different internal implant/abutment connection

Hossam I. Nassar [a,*], Mohamed Farouk Abdalla [b]

[a] Removable Prosthodontics Department, Future University, Cairo, Egypt
[b] Removable Prosthodontics Department, Cairo University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form
29 June 2015
Accepted 6 August 2015
Available online 9 September 2015

Keywords:
Bacterial leakage
Internal connection
Internal hexagonal
Tri-lobe

ABSTRACT

Objectives: This research was carried out to evaluate the bacterial leakage of two different internal implant abutment connections in vitro.

Materials and Methods: Twenty dental implants divided into two equal groups were compared; Group 1 fixtures with an internal hexagonal geometry; Group 2 fixtures with a tri-lobe internal connection. A bacterial suspension of *Staphylococcus aureus* was prepared to obtain a density of 0.5 McFarland standards. All implant abutment assemblies were submerged in sterile tubes containing 4 mL of *S. aureus* broth culture and were incubated at 37 °C for 14 days. The specimens were disassembled and the inner surfaces of the implants were sampled by sterile paper points. Then the paper points were immersed in test tubes containing sterile BHI broth. From the broth, culture was done on blood agar plates and incubated at 37 °C for 24 h. The resulting colonies were identified by Gram's stain and biochemical reactions.

Results: Internal hexagon implants showed statistically significant higher mean Log_{10} CFU than Tri-lobe implants.

Conclusion: Bacterial leakage seems to be inevitable but fixture abutment interface geometry plays an important role in the amount of leakage.

1. Introduction

Microbial leakage at the implant-abutment connection is a chief challenge for the construction of the two-stage implant systems. Gaps and cavities are formed between the implant and the abutment which lead to microbial leakage. This leakage is a major contributing factor for peri-implant inflammatory reactions [1,2].

In the two stage implant placement technique, the implant is placed at the bone crest level and, after 3–6 months, a prosthetic abutment is installed on the implant to connect the implant to future prosthetic restorations (crowns, bridges or dentures), creating a micro-gap between the implant–abutment interface that could present a risk for bacterial colonization [3,4].

The amount of bacterial colonization between the implants and abutments depends on the fit accuracy between the fixture and abutment, their tightening torque and micro-movements between the connected components during mastication [5–8]. The goal of preventing bacterial infiltration

* *Corresponding author*. Tel.: +20 (0)1006049541
E-mail address: hosnassar@hotmail.com (H.I. Nassar).
Peer review under responsibility of Faculty of Oral & Dental Medicine, Future University.
http://dx.doi.org/10.1016/j.fdj.2015.09.001

at the implant abutment interface was to minimize the inflammatory reaction and therefore maintain the bone around the implant top [9,10].

Several attempts to obtain a more secure connection between the abutment base and the implant fixture have been studied. External and internal connections, such as hexagonal, conical (Morse taper) or a combination of both, are generally the most commonly used connections. The internal implants abutment connection is reported as being more favorable to the infiltration of fluids than other joints. The microgap in this implant design varies from 1 to 49 μm, depending on the type of abutment that is selected [11–13].

Different bacterial species with varying sizes from 1 to 10 μm were used in several in vitro studies [6,12,14–17] to detect bacterial infiltration in microgaps. However, biologically small molecules like toxins and molecular constituents of the bacterial wall are responsible for inflammatory reactions. These small molecules can penetrate much smaller gaps than whole bacteria. It is well known that endotoxin, a small molecule complex of lipopolysaccharides and proteins, is one of the most important toxins of gram-negative bacteria and plays a major role in bone destruction processes [11,18,19].

Microleakage has been confirmed to occur in both directions, from the inner parts of the implants to the external environment and vice versa. Reported measures to prevent or minimize bacterial contamination of the implant–abutment interface, such as the use of sealing materials, decontamination of the inner-implant cavity, use of shape memory alloy and different connection geometries, have been unsuccessful [15,20,21].

The purpose of this investigation is to evaluate the microbial leakage of two different internal implant abutment connections in vitro.

2. Material and method

2.1. *Implant experiment groups*

For this study, twenty dental implants (Biocompatible titanium alloy-resorbable blast textured (rbt) body 4.0 × 12 mm) (BioHorizons Implant Systems Inc.) divided into two equal groups were compared based on their fixture abutment interface microgap geometry. Group 1 fixtures with an internal hexagonal geometry were connected to standard straight abutments with a height of 6 mm the abutments were connected to the fixtures with a torque of 25 Ncm according to the manufacturer's protocol; Group 2 fixtures with a tri-lobe internal connection were connected to 3-mm high abutments of 35 Ncm according to manufacturer's recommendation (Fig 1).

2.2. *Preparation of microorganism*

Staphylococcus aureus, identified with Gram's stain and biochemical reactions (catalase and coagulase tests) was used in this study. A bacterial suspension was prepared by cultivating S. *aureus* in brain heart infusion (BHI) broth and incubating it for 24 h at 37 °C. Thereafter, the suspension was diluted in nutrient broth to obtain a density of 0.5 McFarland standards (1×10^8 colony-forming units per milliliter).

3. Microbial sampling and detection

The implants were removed from their packaging under sterile conditions. Subsequently, they were held with sterile pliers to allow a firm torque action and kept in a vertical position. The abutments were carefully connected to the implants according to the manufacturer's instructions. All implant/abutment assemblies were submerged in tubes containing sterile BHI broth for 30 s to determine whether there was any external contamination. The tubes were then incubated at 37 °C for 14 days.

All implant abutment assemblies were submerged in sterile tubes containing 4 mL of *S. aureus* broth culture and were incubated at 37 °C for 14 days. After 14 days of incubation, the specimens were removed from the test tubes using sterile pliers, immersed in 70% alcohol for 3 min to prevent external contamination, and dried with sterile gauze. The specimens were disassembled carefully. After disassembling of the specimens, the inner surfaces of the implants were sampled by sterile paper points for bacterial contamination. Then the paper points were immersed in test tubes containing sterile BHI broth. From the broth, culture was done on blood agar plates and incubated at 37 °C for 24 h. Thereafter, the resulting colonies were identified by Gram's stain and biochemical reactions. Figs. 2–3.

4. Results

Data were presented as mean, median, standard deviation (SD) and range values. Mann–Whitney U test was used to compare between two groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM®[1] SPSS®[2] Statistics Version 20 for Windows.

There was a statistically significant difference between the two groups (*P*-value <0.001). Internal hexagon implants showed statistically significant higher mean Log_{10} CFU than Tri-loaded implants. Table 1.

5. Discussion

The microscopic space between implant and abutment (microgap) facilitates the infiltration of fluids and macromolecules from tissue fluids and saliva, facilitating bacterial invasion and proliferation [4–6], even in patients with good oral hygiene [1,12,20,22,23]. The bacterial contamination may be eventually correlated with gap sizes or misfits. The level of contamination depends not only on the precision of fit, but also on the degree of the applied micromovement and torque. The incidence of loads and unscrewing of the prosthetic abutment can increase infiltration, whereas optimal adaptation, minimal micromovement and exceptional prosthetic

[1] ® IBM Corporation, NY, USA.
[2] ® SPSS, Inc., an IBM Company.

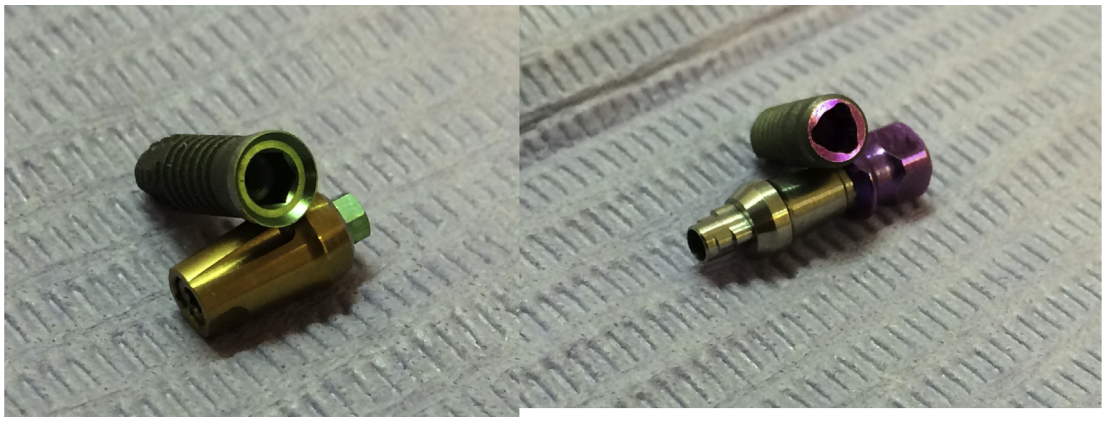

Fig. 1 – Trilobe connection and internal hexagon connection respectively.

Fig. 2 – Implant in BHI broth.

and occlusal planning are factors that can minimize microleakage [8,24].

Several in vitro studies have described the occurrence of bacterial leakage along the implant–abutment interface of systems with different internal connection designs in static or dynamic loading conditions [20,21,25]. Quirynen et al. [26] demonstrated that bacterial invasion of the implant-abutment microgap was detected when fixtures and abutments were assembled and installed in a liquid blood medium inoculated with oral microorganisms. Similarly, Jansen et al. [1] reported microbial leakage of 13 different implant–abutment combinations using E. coli as the indicator bacteria. Callan et al. [27] described moderate to high levels of eight different periodontal pathogenic microorganisms, including A. actinomycetemcomitans and P. gingivalis, colonizing the microgap using DNA-probe analysis. Tesmer et al. [28] assessed the potential risk for invasion of oral microorganisms into the fixture abutment microgap of dental implants with internal Morse-taper connections and the tri-channel internal connection.

Table 1 – Descriptive statistics and results Mann–Whitney U test for comparison between Log_{10} CFU in the two groups.

	Internal hexagon connection	Tri-lobe connection	P-value
Mean (SD)	9.2 (0.1)	8.5 (0.2)	<0.001*
Median (Range)	9.2 (9.1–9.4)	8.6 (8.3–8.7)	

*: Significant at $P \leq 0.05$.

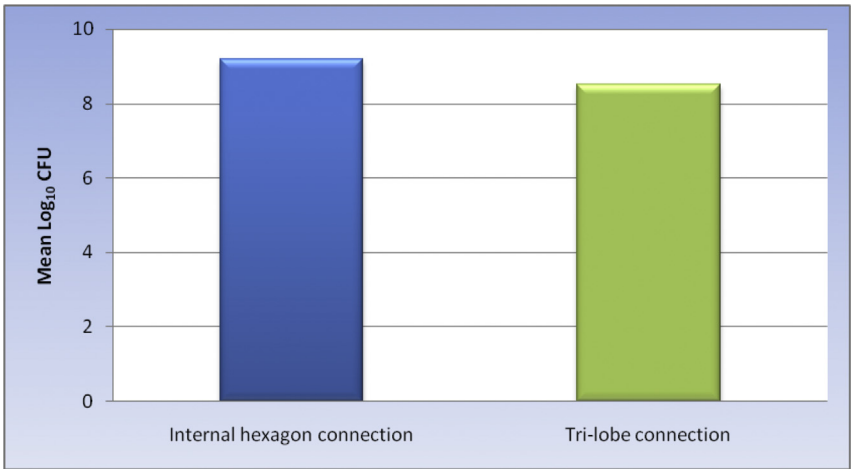

Fig. 3 – Bar chart representing mean Log_{10} CFU in the two groups.

The 14-day period to observe implant external contamination confirms the study by Koka et al. [29] who verified that subgingival bacterial colonization proceeds in the same time interval. Nakazato et al. [30] however, showed that it takes only 4 h for bacterial colonies to be seen on abutment surfaces.

The bacteria infiltration may occur both from an external source to the inner area of an implant and in reverse. This migration of bacteria is probably facilitated through the unavoidable presence of microgaps between the fixture and the abutment components of the assembled system [8,22,31]. The existence of such bacterial leakage is not surprising if one compares the diameter of oral microorganisms (less than 10 μm) with the passive fit between implant components. Binon et al. [32] measured the gap between implants and abutments of different systems and reported dimensions ranging from 20 μm (Implant Innovation) to 49 μm (Nobelpharma AB). The fit between abutment and prosthetic suprastructure shows even larger gaps.

A wide variety of microorganisms seem to be able to penetrate along the implant components, ranging from gram positive cocci to gram-negative rods. Some of the identified species (Bacteroides species, Fusobacterium species and Peptostreptococcus micros) have been associated with periimplantitis [25,27]. Current implant systems cannot completely prevent microbial leakage and bacterial colonization of the inner part of the implant. The penetration of oral microorganisms through the implant abutment interface may produce soft-tissue inflammation and constitute risk to the clinical success of the implants.

Loading forces on implants may also contribute to the bacterial colonization of the fixture abutment interface microgap. One disadvantage of the present in vitro study is that loading conditions were not applied. For instance, in an in vitro experiment using loading forces, Steinebrunner et al. [8] evaluated bacterial leakage along the fixture abutment interface microgap and discovered statistically significant differences between five implant systems with respect to the number of chewing cycles and bacterial colonization. Later Nascimento et al. [33] evaluated bacterial leakage of human saliva under loaded and unloaded using DNA check board. Thus, it is important to confirm or contrast the results of the present study using loading conditions.

6. Conclusion

Bacterial leakage seems to be unavoidable but fixture abutment interface geometry plays an important role in the amount of leakage. Trilobe internal connection showed less amount of bacterial leakage than internal hexagonal connection under static condition.

REFERENCES

[1] Jansen VK, Conrads G, Richter EJ. Microbial leakage and marginal fit of the implant-abutment interface. Int J Oral Maxillofac Implants 1997;12(4):527–40.

[2] Ricci G, Aimetti M, Stablum W, Guasti A. Crestal bone resorption 5 years after implant loading: clinical and radiologic results with a 2-stage implant system. Int J Oral Maxillofac Implants 2004;19(4):597–602.

[3] King GN, Hermann JS, Schoolfield JD, Buser D, Cochran DL. Influence of the size of the microgap on crestal bone levels in non-submerged dental implants: a radiographic study in the canine mandible. J Periodontol 2002;73(10):1111–7.

[4] 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):1372–83.

[5] Byrne D, Houston F, Cleary R, Claffey N. The fit of cast and premachined implant abutments. J Prosthet Dent 1998;80(2):184–92.

[6] do Nascimento C, Barbosa RE, Issa JP, Watanabe E, Ito IY, Albuquerque Jr RF. Bacterial leakage along the implant-abutment interface of premachined or cast components. Int J Oral Maxillofac Surg 2008;37(2):177–80.

[7] Scarano A, Assenza B, Piattelli M, Iezzi G, Leghissa GC, Quaranta A, et al. A 16-year study of the microgap between 272 human titanium implants and their abutments. J Oral Implantol 2005;31(6):269–75.

[8] Steinebrunner L, Wolfart S, Bossmann 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):875–81.

[9] Dibart S, Warbington M, Su MF, Skobe Z. In vitro evaluation of the implant-abutment bacterial seal: the locking taper system. Int J Oral Maxillofac Implants 2005;20(5):732–7.

[10] Ribeiro CG, Maia ML, Scherrer SS, Cardoso AC, Wiskott HW. Resistance of three implant-abutment interfaces to fatigue testing. J Appl Oral Sci 2011;19(4):413–20.

[11] Harder S, Dimaczek B, Acil Y, Terheyden H, Freitag-Wolf S, Kern M. 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(4):427–32.

[12] Ricomini Filho AP, Fernandes FS, Straioto FG, da Silva WJ, Del Bel Cury AA. Preload loss and bacterial penetration on different implant-abutment connection systems. Braz Dent J 2010;21(2):123–9.

[13] da Silva-Neto JP, Nobilo MA, Penatti MP, Simamoto Jr PC, 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.

[14] Barbosa RE, do Nascimento C, Issa JP, Watanabe E, Ito IY, de Albuquerque Jr RF. Bacterial culture and DNA checkerboard for the detection of internal contamination in dental implants. J Prosthodont 2009;18(5):376–81.

[15] Duarte AR, Rossetti PH, Rossetti LM, Torres SA, Bonachela WC. In vitro sealing ability of two materials at five different implant-abutment surfaces. J Periodontol 2006;77(11):1828–32.

[16] do Nascimento C, Barbosa RE, Issa JP, Watanabe E, Ito IY, de Albuquerque Junior RF. Use of checkerboard DNA-DNA hybridization to evaluate the internal contamination of dental implants and comparison of bacterial leakage with cast or pre-machined abutments. Clin Oral Implants Res 2009;20(6):571–7.

[17] do Nascimento C, Pedrazzi V, Miani PK, Moreira LD, de Albuquerque Jr RF. Influence of repeated screw tightening on bacterial leakage along the implant-abutment interface. Clin Oral Implants Res 2009;20(12):1394–7.

[18] Coelho PG, Sudack P, Suzuki M, Kurtz KS, Romanos GE, Silva NR. In vitro evaluation of the implant abutment

connection sealing capability of different implant systems. J Oral Rehabil 2008;35(12):917–24.
[19] Besimo CE, Guindy JS, Lewetag D, Meyer J. Prevention of bacterial leakage into and from prefabricated screw-retained crowns on implants in vitro. Int J Oral Maxillofac Implants 1999;14(5):654–60.
[20] 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):328–35.
[21] Pautke C, Kolk A, Brokate M, Wehrstedt JC, Kneissl F, Miethke T, et al. Development of novel implant abutments using the shape memory alloy nitinol: preliminary results. Int J Oral Maxillofac Implants 2009;24(3):477–83.
[22] Rimondini L, Marin C, Brunella F, Fini M. Internal contamination of a 2-component implant system after occlusal loading and provisionally luted reconstruction with or without a washer device. J Periodontol 2001;72(12):1652–7.
[23] Dias EC, Bisognin ED, Harari ND, Machado SJ, da Silva CP, Soares GD, 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(2):346–51.
[24] Harder S, Quabius ES, Ossenkop L, Kern M. Assessment of lipopolysaccharide microleakage at conical implant-abutment connections. Clin Oral Investig 2012;16(5):1377–84.
[25] Cosyn J, Van Aelst L, Collaert B, Persson GR, De Bruyn H. The peri-implant sulcus compared with internal implant and suprastructure components: a microbiological analysis. Clin Implant Dent Relat Res 2011;13(4):286–95.
[26] Quirynen M, Bollen CM, Eyssen H, van Steenberghe D. Microbial penetration along the implant components of the branemark system. An in vitro study. Clin Oral Implants Res 1994;5(4):239–44.
[27] Callan DP, Cobb CM, Williams KB. DNA probe identification of bacteria colonizing internal surfaces of the implant-abutment interface: a preliminary study. J Periodontol 2005;76(1):115–20.
[28] Tesmer M, Wallet S, Koutouzis T, Lundgren T. Bacterial colonization of the dental implant fixture-abutment interface: an in vitro study. J Periodontol 2009;80(12):1991–7.
[29] Koka S, Razzoog ME, Bloem TJ, Syed S. Microbial colonization of dental implants in partially edentulous subjects. J Prosthet Dent 1993;70(2):141–4.
[30] Nakazato G, Tsuchiya H, Sato M, Yamauchi M. In vivo plaque formation on implant materials. Int J Oral Maxillofac Implants 1989;4(4):321–6.
[31] Piattelli A, Scarano A, Paolantonio M, Assenza B, Leghissa GC, Di Bonaventura G, et al. Fluids and microbial penetration in the internal part of cement-retained versus screw-retained implant-abutment connections. J Periodontol 2001;72(9):1146–50.
[32] Binon P, Weir D, Watanabe L, Walker L. In: Laney W, Tolman D, editors. Implant component compatibility. Tissue integration in oral orthopedic & maxillofacial reconstruction. Chicago: Quintessence Publishing; 1992. p. 218–26.
[33] do Nascimento C, Miani PK, Pedrazzi V, Goncalves RB, Ribeiro RF, Faria AC, 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):551–60.