Bacterial Leakage at Implant-Abutment Interface with Different Intermediate Materials

Farnoush Mohammadi, DDS, MSc[1]
Maryam Hajmousaei[2]
Nastaran Vaziri[3]
Mahnaz Arshad[4]*

A gap exists at the implant-abutment interface in two-piece implants and can serve as a reservoir of bacteria and compromise the health of peri-implant tissue. This study aimed to compare the effect of different intermediate materials on bacterial leakage at the implant-abutment interface. A total of 75 implants were divided into 5 groups (n = 15) based on the material applied at the implant-abutment connection: (1) Atridox, (2) chlorhexidine, (3) Gapseal silicone, (4) saliva, and (5) no material. All the implants were inoculated with 0.1 μL of Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans) suspension, and then the respective material was applied. The abutments were connected to the implants, and appropriate torque was applied as recommended by the manufacturer (Implantium, Dentium, Korea, Seoul). Bacterial leakage was determined by evaluating the turbidity of the broth. Bacterial contamination was found in all samples at different times; in groups 1, 2 and 3, contamination was noted after 7, 5, and 6 days, respectively, on average. Contamination occurred averagely after 4 days in groups 4 and 5. The present study showed that Atridox applied at the implant-abutment interface significantly delayed bacterial leakage.

Key Words: dental implant-abutment interface, bacterial leakage, implant

INTRODUCTION

At present, intraosseous titanium implants are commonly used to replace the lost teeth. One- and two-piece implants are available in the market.[1] Precise fit between the implant and abutment in two-piece implants is important for their long-term durability.[2] Gaps are present at the implant-abutment interface and also between the threads and the screw hole.[3] These gaps serve as a trap for bacteria.[4] Internal contamination of implant can occur in two ways:

1. When an abutment is connected to an implant, gaps between the components are unavoidable.[5]
2. Bacteria enter the inner part by leakage through the implant-abutment interface.[6]

Several studies have reported inward and outward leakage of bacteria, oral fluids, and nutrients through the implant-abutment interface. After colonization, bacteria can invade the peri-implant tissue and cause peri-implantitis.[7] Also, in two-piece implants, the peri-implant crestal bone level depends on the location of the implant-abutment interface (microgaps).[8] Infiltration of inflammatory cells can lead to peri-implantitis and eventual bone loss.[9] Bacterial infection also interferes with osseointegration during the healing phase.[10] Thus, it is a challenge for researchers and implant manufacturers to decrease the size of microgaps and achieve an ideal fit.[11]

Several studies have attempted to access and minimize microleakage through the implant-abutment interface.[12]

Torres et al used a new nitrogen flow technique to measure the amount of microleakage through the implant-abutment interface. They found that there are significant differences between different sealing and screwing conditions.[13] Assenza et al studied 3 implant connections—namely, cement-retained, internal conical, and screw-retained—and confirmed the results of previous studies regarding the hermiticity of cement-retained implant-abutment assembly, low bacterial penetration in internal conical connection and high prevalence of bacterial leakage in screw-type connection.[4] Koutouzis et al evaluated endotoxin penetration through the implant-abutment interface using chlorhexidine solution at the interface.[14] Ghannad et al found that the application of 1% chlorhexidine gel decreased bacterial growth at the implant-abutment interface.[15] Chadha et al[16] evaluated doxycycline controlled-release gel versus doxycycline controlled-release implant for management of periodontitis. The authors found that compared with scaling alone, the use of doxycycline (whether gel or implant) had a greater efficacy for improvement of periodontal condition. Atridox is a controlled-release doxycycline gel that is applied

[1] Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran; Department of Oral and Maxillofacial Surgery, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.
[2] DDS, Tehran University of Medical Sciences, Tehran, Iran.
[3] Dental School Student, School of Dentistry, International Campus, Tehran University of Medical Sciences, Tehran, Iran.
[4] DDS, MSc, Assistant Professor, Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran; Department of Prosthodontics, School of Dentistry, International Campus, Tehran University of Medical Sciences, Tehran, Iran.
* *Corresponding Author*, e-mail: mahnazarshad@yahoo.com
https://doi.org/10.1563/aaid-joi-D-18-00313

subgingivally. It affects periodontal pathogens and improves the periodontal condition.[16]

Reliable guidelines or standardized surgical protocols have not been provided by the manufacturers regarding the use of antibacterial agents at the implant-abutment interface, which forces the clinicians to come up with methods to minimize bacterial leakage.[17]

Although many studies have attempted to decrease leakage at the implant-abutment interface using different materials from saline to antibiotics, no study has compared the efficacy of these materials to prevent bacterial leakage.[18,19]

The aim of this study was to compare the efficacy of 3 materials used at the implant-abutment interface in the presence and absence of saliva. The following hypotheses were tested:

(H1) Reduction of bacterial leakage by using Atridox at the implant-abutment junction in comparison with not using it, is more.
?1
(H2) Reduction of bacterial leakage by using Atridox at the implant-abutment junction in comparison with using silicon in this space, is more
(H3) Reduction of bacterial leakage by using Atridox at the implant-abutment junction in comparison with using chlorhexidine in this space, is more.
(H4) Reduction of bacterial leakage by using Atridox at the implant-abutment junction in comparison with the existence of saliva in this space, is more.

Materials and Methods

A total of 75 Superline fixtures (12 mm height, 4 mm diameter) and 75 abutments were used in this in vitro study. The minimum sample size was calculated to be 12 samples in each group according to a study by Assenza et al. The methodology was reviewed by an independent statistician.

Fixtures were randomly divided into 5 groups ($n = 15$). Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans) was used in this study. A. actinomycetemcomitans is a gram-negative, facultative/anaerobic bacterium found in the oral cavity. It is considered an oral commensal measuring about 0.4×1.0 μm. It can cause periodontitis and peri-implantitis. To prepare a bacterial suspension, A. actinomycetemcomitans was plated in tryptic soy agar yeast plates and incubated at 37°C for 48 hours in presence of 0.5% CO_2. A few bacterial colonies were diluted in tryptic soy broth supplemented with yeast extract (TSBY) to a density of 0.5 McFarland standard concentration (1×10^8 colony forming units/mL), confirmed by spectrophotometric analysis.

The sensitivity of A. actinomycetemcomitans to Atridox, chlorhexidine, and Gapseal silicone was then evaluated. For this purpose, 3 plates of tryptic soy agar yeast were prepared, and their surfaces were inoculated with bacteria. Then, one drop of each material was placed on each plate and incubated at 37°C for 48 hours in the presence of 0.5% CO_2. The results indicated that bacterial colonies did not grow around Atridox and chlorhexidine but grew normally around Gapseal silicone. This result confirmed the sensitivity of A. actinomycetemcomitans to Atridox and chlorhexidine.

The inner part of all implants was then inoculated with 0.1 μL of viable A. actinomycetemcomitans with sterile instruments under sterile conditions. The volume of the inner part of the fixture for each material was precisely measured.

After bacterial inoculation, the implants were divided into 5 groups, and each group was inoculated with 4.9 μL of respective materials: (1) Atridox (10% doxycycline hyclate, TOLMAR Inc, Fort Collins, Colo), (2) chlorhexidine (Colgate, New York. NY), (3) Gapseal silicone (Hager & Werken GmbH & Co KG, Duisburg, Germany), (4) saliva and (5) no material.

In all groups, the abutments were carefully connected to fixtures using sterile gloves and torqued according to the manufacturer's recommendations regarding the closing torque (Implantium, Dentium, Seoul, Korea). An electronic torquemeter was used to ensure proper seating torque for all implants. As a positive control, 2 test tubes were filled with TSBY only and inoculated with 0.1 μL of A. actinomycetemcomitans. The turbidity of broth confirmed the viability and growth of microorganisms in this study.

Two test tubes were filled with TBSY only and served as negative controls. These tubes showed a transparent solution with no bacterial growth.

The entire assembly was immersed in TSBY in a rolling motion to evaluate inadvertent contamination of the external surface; tubes showing turbidity of broth (indicating colonization and contamination of surface) were excluded. The samples were then placed in tubes; the amount of nutrient solution required in the tubes was determined exactly such that the level of fluid remained right above the implant-abutment interface. All the procedures were performed under a laminar flow hood (Class B, Jal Tajhiz, Iran). The tubes containing samples, the test tubes used for external contamination testing, the negative control tubes, and the positive control tubes were incubated at 37°C in the presence of 5% CO_2 for 28 days.

The broth culture of the vials containing samples was refreshed every 7 days. Any possible penetration of bacteria was determined by evaluating the turbidity of the broths. Samples were checked daily, and the presence/absence of turbidity was recorded. Leakages cause the colonization of bacteria, and the colonization of bacteria causes cloudiness and turbidity of the solution (Figure 1). Next, 1 μL of the cloudy solution was analyzed for the morphology of colonies in tryptic soy agar yeast plate incubated at 37°C for 48 hours to confirm the purity of bacteria used to inoculate the inner part of the implant. The growth and proliferation of A. actinomycetemcomitans showed that the bacteria had moved from the inner part of the implant into the surrounding solution through the implant-abutment interface. The experiment was repeated for some implants showing external surface contamination.

Statistical analysis

Statistical analysis was done using SPSS software version 24. The differences between the groups were statistically analyzed by Kruskal-Wallis and Bonferroni adjusted Dunn's multiple comparisons test. Statistically significant differences were accepted at $P < 0.05$.

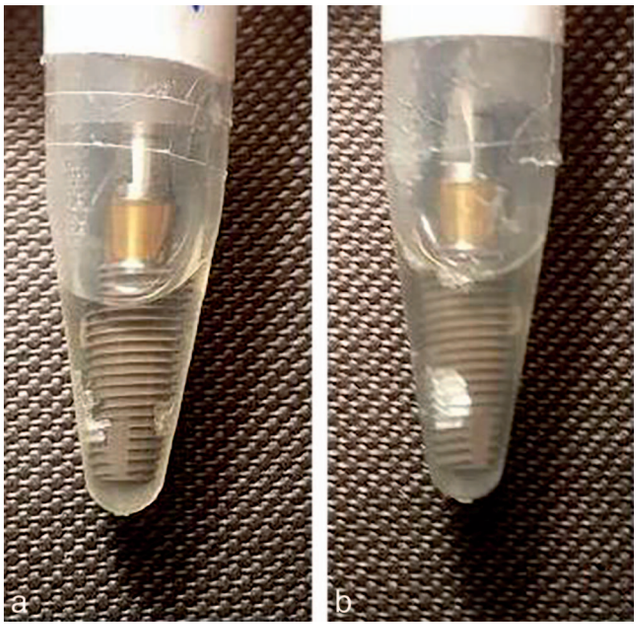

Figure 1. Implants of group 2 placed in the nutrition solution. (a) No contamination. (b) Cloudiness of broth indicative of bacterial contamination.

Results

In this study, bacterial contamination was found in all samples, with differences in the distribution of time of occurrence among 5 groups ($P = 0.004$). Descriptive statistics for the day of bacterial occurrence is given in Table 1. Post hoc comparisons revealed significant differences between Atridox with saliva and no material ($P = 0.008$ and $P = 0.025$).

The cumulative proportion of contaminated samples during the study period is given in Figure 2 for all of the studied subgroups.

Discussion

Gaps between the components in 2-piece implant systems can serve as a trap for bacteria. Previous studies have demonstrated that infiltration of inflammatory cells around implants is related to microleakage through the implant-abutment interface. This inflammatory process in soft and hard tissues around the implant interferes with its long-term durability. Contamination of the inner part of the implant during the prosthetic phase is often inevitable and causes bacterial colonization at the implant-abutment interface. Bacterial colonization close to the bone may lead to inflammation and eventual bone loss. This is one of the most important factors causing 1 mm of bone loss in the first year of implant placement. Bacterial leakage through the implant-abutment interface causes an immune reaction and periodontal inflammation.[20,21] Torres et al found that sealing of the implant-abutment interface can decrease microleakage.[13] Rimondini et al[22] demonstrated that bacterial microleakage occurs in clinical conditions; applying a silicone washer at the implant-abutment interface can decrease bacterial leakage, but very good oral hygiene is still more effective in reducing contamination. While Rimondini and colleagues demonstrated that sealing of the implant-abutment interface with Gapseal could not prevent bacterial leakage, it could inconsiderably delay the occurrence of leakage.[22] Mehl et al compared three sealing agents—Gapseal, AGC Cem, and Cervetic Plus—to prevent microleakage in prefabricated 2-implant bar attachment system.[23] The results indicated that microleakage initially occurred in some groups, but after 1000 loading cycles, microleakage was observed in all groups, and none of the sealing agents could prevent microleakage. In our study, even applying Gapseal at the implant-abutment interface could not completely prevent bacterial leakage.

Table 1

Descriptive statistics for the occurrence day of bacterial contamination

Group	Mean	SD	Median	IQR	Min	Max
Atridox	7.1	0.4	7	(7,7)	7	8
Chx	5.3	1.4	5	(5,5)	2	8
Silicon	6.7	2.4	5	(5,8)	5	12
Saliva	4.6	4.9	1	(1,11)	1	13
No Material	4.6	3.4	3	(1,6)	1	12

Studies on the use of chlorhexidine found that 0.2% chlorhexidine rinse is effective for reduction of the count of oral microflora and bacterial pathogens.[24] Paolatinio et al evaluated the efficacy of chlorhexidine when used at the implant-abutment interface and found that applying chlorhexidine to the inner part of the fixture decreased bacterial colonization.[25] However, Koutouzis et al demonstrated that bacterial toxins leaked through the implant-abutment interface despite the use of chlorhexidine.[14] The present study confirmed the findings of the 2 studies previously mentioned. In our study, A. actinomycetemcomitans was sensitive to chlorhexidine, but applying chlorhexidine to the implant-abutment interface could not prevent bacterial leakage. However, it caused an insignificant delay in the time of occurrence of leakage.

Mombelli et al found that local delivery of tetracycline improved the clinical periodontal parameters.[26] Park et al used tetracycline with deproteinized bovine bone to treat peri-implantitis and report its positive results.[27] Chadha et al evaluated doxycycline controlled-release gel versus doxycycline controlled-release implants and found that local doxycycline, whether gel or implant and in comparison with only scaling and root planing, had higher efficacy for treatment of periodontal disease. There was no significant difference between gel and implant.[16]

Atridox, chlorhexidine, and Gapseal could not completely prevent bacterial leakage but when compared to the presence of saliva or no treatment at the implant-abutment interface, they significantly delayed the occurrence of leakage. To the best of the authors' knowledge, no previous in vitro study has used Atridox at the implant-abutment interface, but the present study has confirmed the available data reported in the literature.

According to this study, the use of Atridox gel is recommended for patients; it significantly delays bacterial leakage and decreases crestal bone resorption.

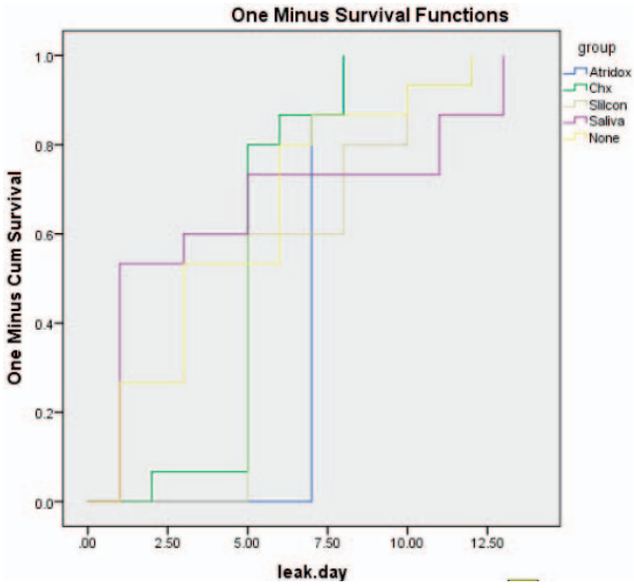

Figure 2. The cumulative proportion of samples with bacterial contamination over study period in studied subgroups.

This study had an in vitro design; thus, in vivo studies are still required on this topic. To assess the effect of occlusal forces on screw loosening, cyclic loading is important and should be performed. Screw loosening creates a space between the implant and abutment and also increases the secretion of bacterial endotoxins due to pumping movements. Therefore, it is recommended to perform cyclic loading and assess its effects on results in future studies. The lower the limitations of studies, the more realistic the results will be.

Conclusion

Three materials that are routinely used to seal the implant-abutment interface were compared in this study. Atridox and chlorhexidine have confirmed antibacterial effects. Atridox seals the implant-abutment interface and limits bacterial accumulation and endotoxin production. There is no evidence of research done on Gapseal silicone or comparing the above-mentioned 3 materials. Further, presence and absence of saliva at the implant-abutment interface were compared in our study. This comprehensive study compared the effects of the above-mentioned 3 materials on bacterial growth and crestal bone resorption.

- The present study confirmed previous results about the use of Atridox, showing that it can prevent bacterial colonization for a period of time. Bacterial leakage always occurs, but Atridox caused a significant delay in the onset of leakage when used at the implant-abutment interface.
- Presence/absence of saliva did not have any significant effect on the time of onset of bacterial leakage.
- This study showed that chlorhexidine and Gapseal did not prevent bacterial leakage but caused an inconsiderable delay in the onset of leakage.

Abbreviation

TSBY: tryptic soy broth supplemented with yeast

Acknowledgments

The authors thank Dentium Company (Seoul, South Korea) for supplying implant components for evaluation, and Hager and Werken Co (Duisburg, Germany) for supplying Gapseal. The authors would also like to thank Dr Amir Ali-Ramezani and Dr Masoumeh Doraghi, Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, as well as Dr Mohammad Javad Kharazifard at the Department of Epidemiology of Tehran University of Medical Sciences for statistical analyses. Further thanks to Dr Mahshid Namdari (Shahid Beheshti University of Medical Sciences) for reviewing the statistics part, methodology, result, and conclusion.

Note

The authors declare no conflicts of interest.

References

1. do Nascimento C, Miano 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 Dent*. 2012;27:551–560.

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

3. Gross M, Abarmovich I, Weiss EI. Microleakage at the abutment-implant interface of osseointegration implant: a comparative study. *Int J Oral Maxillofac Implants*. 1999;14:94–100.

4. 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.

5. Jansen VK, Conrads G, Richter E-J. Microbial leakage and marginal fit of the implant-abutment interface. *Int J Oral Maxillofac Implants*. 1997;12:527–540. Erratum in: *Int J Oral Maxillofac Implants*. 1997;12:709.

6. do Nascimento C, Barbosa R, Issa J, Watanabe E, Ito I, Albuquerque R. Bacterial leakage along the implant-abutment interface of premachined or cast components. *Int J Oral Maxillofac Surg*. 2008;37:177–180.

7. 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;20:732–737.

8. Hermann JS, Buser D, Schenk RK, Schoolfield JD, Cochran DL. Biologic Width around one and two piece titanium implants. 2001;12:559–571.

9. Broggini N, McManus L, Hermann JS, et al. Peri-implant inflammation defined by the implant-abutment interface. *J Dent Res*. 2006;85:473–478.

10. Arshad M, Siadat H, Fallahi B, Mahgoli H. Determination of a proper covering material for fixture-abutment microleakage evaluation using radiotracers and gamma-counter; a pilot study. *J Islam Dent Assoc Iran*. 2013;25:15–21.

11. 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;14:654–660.

12. Andreasi Bassi M, Lopez MA, Confalone L, Gaudio RM, Lombardo L, Lauritano D. A prospective evaluation of outcomes of two tapered implant systems. *J Biol Regul Homeost Agents*. 2016;30:1–6.

13. Torres JH, Mechali M, Romieu O, et al. Development of a new quantitative gas permeability method for dental implant-abutment connec-

tion tightness assessment. *Biomed Eng Online*. 2011;10:28. https://doi.org/10.1186/1475-925X-10-28

14. Koutouzis T, Gadalla H, Kettler Z, Elbarasi A, Nonhoff J. The role of chlorhexidine on endotoxin penetration to the implant-abutment interface (IAI). *Clin Implant Dent Relat Res.* 2015;17:476–482.

15. Ghannad F, Alkadi LT, Wiebe CB, Shen Y, Haapasalo M, Larjava HS. Intra-operative application of chlorhexidine gel reduces bacterial counts in internal implant cavity. *Eur J Oral Sci.* 2015;123:425–431.

16. Chadha VS, Bhat KM. The evaluation of doxycycline controlled release gel versus doxycycline controlled release implant in the management of periodontitis. *J Indian Soc Periodontol.* 2012;16:200–206.

17. Tesmer M, Wallet S, Koutouzis T, Lundgren T. Bacterial colonization of the dental implant fixture–abutment interface: an in vitro study. *J Periodontol*. 2009;80:1991–1997. https://doi.org/10.1902/jop.2009.090178

18. Sönke H, Birka D, Yaha A, Hendrik T, Sandra FW, Matthias K. Molecular leakage at implant-abutment connection – in vitro investigation of tightness of internal conical implant-abutment connections against endotoxin penetration. *Clin Oral Invest*. 2010;14:427–432. https://doi.org/10.1007/s00784-009-0317-x

19. 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;35:917–924. https://doi.org/10.1111/j.1365-2842.2008.01886.x

20. 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;72:1372–1383.

21. Adell R, Lekholm U, Rockler B, et al. Marginal tissue reactions at osseointegrated titanium fixtures (I). A 3-year longitudinal prospective study. *Int J Oral Maxillofac Surg*. 1986;15:39–52.

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;72:1652–1657.

23. Mehl CJ, Steiner M, Ludwig K, Kern M. Wear, microleakage and plastic deformation of an implant-supported chair-side bar system. *J Adv Prosthodont*. 2015;7:323–328.

24. Schiött CR, Löe H, Jensen SB, Kilian M, Davies R, Glavind K. The effect of chlorhexidine mouthrinses on the human oral flora. *J Periodontal Res*. 1970;5:84–89.

25. Paolantonio M, Perinetti G, D'Ercole S, et al. Internal decontamination of dental implants: an in vivo randomized microbiologic 6-month trial on the effects of a chlorhexidine gel. *J Periodontol*. 2008;79:1419–1425.

26. Mombelli A, Feloutzis A, Brägger U, Lang NP. Treatment of peri-implantitis by local delivery of tetracycline. Clinical, microbiological and radiological results. *Clin Oral Implants Res*. 2001;12:287–294.

27. Park JB. Treatment of peri-implantitis with deproteinised bovine bone and tetracycline: a case report. *Gerodontology*. 2012;29:145–149.

Queries for orim-45-06-11

1. **Author: Unclear re: “is more”? Do you mean “XXX is better in comparison with not using it”? Please clarify your meaning in these phrases. Copy editor**