Original Article

The effect of dynamic loading on bacterial microleakage of the dental implant fixture-abutment interface: An *in vitro* study

Mehrak Amjadi, Azadeh Khajavi, Navid Youssefi, Negar Barjini, Zahra Kazemi[1]

Department of Prosthodontics, Dental Caries Prevention Research Center, Qazvin University of Medical Sciences, [1]Department of Prosthodontics, Student Research Committee, Qazvin University of Medical Sciences, Qazvin, Iran

Abstract

Aim: Bacterial micro leakage at implant-abutment interface under functional loading is an important factor, may lead to crestal bone loss and affect the long term success of dental implants. Due to the limited studies about the implant systems with a connection of Slip joint design, this study aimed to evaluate the effect of dynamic loading on bacterial leakage at the implant-abutment interface with slip joint connection.

Settings and Design: *In vitro*- comparative study.

Materials and Methods: A total of 20 implants and abutments with slip joint connections (Tapered Screw-Vent, 3.7 mm × 10 mm, Zimmer Dental, USA) was examined and depends on using functional loading were divided into two groups; loaded and unloaded. Initially, 10 µl of Brain Heart Infusion (BHI) culture broth was pipetted to the internal lumen of each implant, then the abutments were tightened to the fixtures and crowns were cemented. In the unloaded group, samples were immersed in E-Coli suspension for 5 days. In the loaded group, samples were immersed in microbial suspension under 500000 cycles using a cyclic load device. Following disconnection of fixtures and abutments, microbial samples were taken from the internal lumen of implants and colonies were counted. Data were analyzed using.

Statistical Analysis Used: Mann-Whitney statistical test, SPSS version 24.

Results: The mean rate of micro leakage in unloaded and loaded groups was 4000 CFU/ml and 27000 ± 31640 CFU/ml respectively. Bacterial colonies grew in 10% of unloaded samples and 50% of loaded samples. This difference was statistically significant. ($P < 0.05$)

Conclusions: Microbial micro leakage at the implant-abutment interface with slip joint design increased significantly after functional loading.

Keywords: Bacterial count, dental implant loading, dental implant-abutment interface, microleakage

Address for correspondence: Dr. Azadeh Khajavi, Department of Prosthodontics, Dental Caries Prevention Research Center, Qazvin University of Medical Sciences, Bahonar Blvd, Qazvin, Iran.
E-mail: azadeh_khajavi@yahoo.com

Submitted: 17-Mar-2021 **Revised:** 16-Aug-2021 **Accepted:** 02-Oct-2021 **Published:** 09-Nov-2021

INTRODUCTION

Prevention of microleakage at implant-abutment interface to minimize the inflammatory response and enhance the marginal bone stability has been a great challenge in two pieces dental implants production.[1] Microorganisms can grow in spaces between implant-abutment interface, called

Access this article online	
Quick Response Code:	Website: www.j-ips.org
	DOI: 10.4103/jips.jips_96_21

How to cite this article: Amjadi M, Khajavi A, Youssefi N, Barjini N, Kazemi Z. The effect of dynamic loading on bacterial microleakage of the dental implant fixture-abutment interface: An *in vitro* study. J Indian Prosthodont Soc 2021;21:420-4.

micro gap, and leak through the surrounding tissues under functional loading. Actually, occlusal forces can make a bacterial pumping between the implant and surrounding tissues through the bending and micro-movement of the implant system and changing the micro gap size.[2,3]

The rate of microbial leakage at the implant-abutment connection depends on the precision of the implant-abutment interface, the strength of the connection, and the components micro movement under loading condition.[4,5]

Different designs of implant-abutment interface in different implant systems influence the precision amount of the connection and its susceptibility to microleakage during functional loading.[5,6] In fact, the implant-abutment interface geometry can affect the abutment stability during the loading. According to the previous studies, this stability in fixtures with internal connection was more than the external ones and could prevent the microbial leakage more efficiently.[7]

Today, various implant systems with internal connection have being used widely in clinic.

Different structural designs of implant-abutment interface near to the crestal bone, can be seen in these implant systems, depends on the profile properties or the type of settlement of external surfaces of the components to each other, including conical, butt joint and slip joint interface. In words, these surfaces in conical design entering to each other and make an acute angle, while in slip joint and butt joint designs, meet each other with obtuse and right angle, respectively.[8]

Abundant studies have been evaluated the rate of microbial leakage at implant-abutment interface with profile of conical and butt joint design and mostly founded that implant systems with conical interface have been more stable mechanically and more successful clinically.[9] There are limited articles that evaluated implants with slip-joint interface in comparison to the other connection designs. In an only study which can be found related to these kinds of interface, it had been reported the less microleakage of the implants with slip-joint design than Conical ones under static condition.[10]

Considering that microbial leakage is one of the important factors in assessing the precision fit and quality of implant-abutment interface[3] and duo to the little information about the implant systems with slip-joint interface, We choose an implant system with that design (Tapered Screw-Vent, Zimmer Dental, USA) for evaluation in this study.

However, there have been various results among the studies which have evaluated the effect of the loading factor on microleakage at implant-abutment interface of a unique implant system. In which, some of them have found that cyclic loading had no effect on the rate of microleakage,[3,11] whereas in some others it was different.[12-14] Hence, the present study evaluated the effect of dynamic loading on the bacterial leakage of dental implants with slip joint connection. The null hypothesis was that the microleakage rate in implant system with slip joint connection under loading condition, would not be changed.

MATERIALS AND METHODS

The study was approved by institutional review board IR.QUMS.REC.1397.110. In this *in vitro* experimental study, 20 implants and abutments with slip-joint connections (Tapered Screw-Vent, 3.7 mm × 10 mm, Zimmer Dental, USA) were selected. This study did not involve humans and there is no need to provide ethical authorization.

Twenty stainless-steel cylindrical chambers were custom made and filled with auto-polymerizing resin (Luxatemp, DGM, Hamburg, Germany). Using a dental surveyor, each implant was vertically embedded into the auto-polymerizing resin in a cylindrical chamber. The implant platform was set approximately 1 mm above the resin level to allow the bacterial penetration assay. Twenty full metal crowns with the first mandibular molar contour were prepared using CADCAM (rainbow TM, Korea). The abutments were then removed from the fixtures, and all abutments, mounted fixtures, crowns, and instruments were sterilized by autoclave.[2]

While preserving the sterilization of the samples, 10 μl of brain heart infusion medium was placed at the deepest point of each implant.[1,15] The abutments were connected to the implants, and their screws were fastened by a torque of 35 Ncm as the manufacturer recommended. Then, to prevent microleakage from the abutment screw hole, the area was completely sealed with gutta-percha (Pumadent, China) and the cyanoacrylate adhesive (Razi Chemical Co., Iran). The crowns were temporarily cemented to the abutments (Temp Bond, Kerr).[16]

As a negative control, three implants from each group were randomly selected and sampling was done from the outer surface of the implants with mico-brush. The samples were cultured in MacConkey agar medium and were incubated for 24 h in an incubator (Memert, Germany) at 37°C.[1,15]

In this study, the inward bacterial leakage of *Escherichia coli* was evaluated. 0.5 ml McFarland trypticase soy broth solution of *E. coli* was prepared in the concentration of 1.5×10^8 CFU/ml.[17-21]

For the first study group (unloaded group), the abutment-crown complex was merged in the *E. coli* suspension for 5 days. The microbial solution surrounding the abutment-crown complex was recharged every 24 h.[1]

For the second group (loaded group), the abutment-crown complex was immersed in the *E. coli* suspension while a cyclic load was applied to the central fossa of each crown with a round stainless-steel stylus. A force of 120 N at a frequency of 1 Hz was applied for a total of 500,000 cycles, equivalent to approximately 2 years of clinical function, and the microbial solutions were recharged every 24 h during the process [Figure 1].[1]

After the 5th day, to ensure that bacteria had been alive during the test, a sample was collected from three random microbial solutions surrounding the complex and cultured on MacConkey agar medium as a positive control.[1]

After 5 days, the outer surface of the samples in the unloaded and loaded groups was disinfected with 70% alcohol, and crowns and abutments were removed while preserving the sterilization. Then, the internal lumen of implants was sampled using a sterile micro-brush and cultured on a MacConkey agar medium. These plates were incubated at 37°C, and after 24 h colony count was performed [Figure 2].[1,15]

The collected data were imported to Statistical Package for Social Sciences (SPSS) for Windows software, version 24.0 (IBM Corp., Armonk, NY, USA). The standard descriptive methods, such as the mean and standard deviation, were applied to determine the characteristics of the sample. Because the distribution of the data was not normal, the nonparametric Mann–Whitney U test was used for the pairwise comparisons of the data. The confidence interval was set to 95% and $P < 0.05$ was considered statistically significant.

Figure 1: Microbial solution recharge during cyclic load

RESULTS

The studied specimens consisted of 20 implants and abutments with slip joint connection which were divided into two groups of 10. No study sample was excluded from the study.

The mean rate of microleakage was 4000 CFU/ml in the unloaded group and 27,000 ± 31,640 CFU/ml in the loaded group. Bacterial colonies grew in 10% (1 sample) of unloaded samples, while the colonies were observed in 50% (5 samples) of loaded samples. This difference was statistically significant ($P < 0.05$). So that, the bacterial microleakage was higher in the loaded group compared to the unloaded group.

DISCUSSION

In the present study, the effect of dynamic loading on microbial microleakage at implant-abutment interface with a design of slip joint connection was evaluated.

According to the literature review, depends on the direction of leakage there are totally two different methods in order to evaluate the microbial leakage at implant-abutment interface in *in vitro* studies, including inward and outward methods.[17-20,22-24] In inward method, microleakage happens from outside toward the internal space of implant and are proved by the bacterial present in the internal lumen of the implant, while in outward way, this procedure is opposite. To prevent the contamination which may occur during abutment attach and detaching from the fixture, lead to a false positive result, the microbial leakage can be assessed only once in inward method. Hence, it would not be possible to trace the rate of microleakage may happen along the time, as the outward method.[24] However, in this study, the inward method was used, because it is a better simulation of the oral environment than the outward method.[10] In this study, the implant-abutment-crown assemblies were placed in the microbial suspension to evaluate the microbial leakage at static condition or under cyclic loading, and sampling from the internal lumen of implants was done after 5 days.[1]

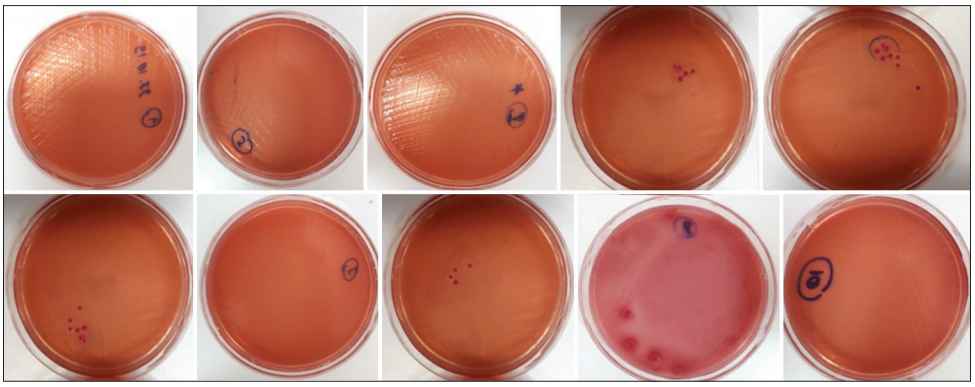

Figure 2: Cultivation in MacConkey agar medium

In the present study, *E. coli* was used to evaluate bacterial leakage. It is a Gram-negative bacillus bacteria with a diameter of 1.1–1.5 μm. Considering the mean measured micro gap size in dental implants under static and loading condition (0.97 μm), so it has the ability to leak from the implant-abutment connection.[25] *E. coli* is a facultative anaerobic bacterium, makes it possible to survive in inappropriate conditions like the implant's internal cavity which may have limited food supply and oxygen for 5 days.[22] Furthermore, it is a bacteria commonly seen in peri-implantitis lesions and has been used in many *in vitro* studies of implant microleakage.[1,24]

Various studies have investigated microbial microleakage at the implant-abutment interface with different connection geometries. It has been shown, the implants with a connection of butt joint design has less mechanical stability and more microleakage at their interface than ones with conical design.[9] On the other hand, there are limited information about the microleakage of implants with slip-joint design at their interface. Manufacturers of implants with slip joint design claim that this interface consists of an internal bevel that starts from the outer part of the platform and extends to the internal hex surfaces of the anti-rotation part of the fixture, leading to abutment stability, reduced microleakage, and better horizontal stress distribution compared to butt joint connections. However, studies that evaluated the accuracy of implant-abutment slip-joint connection and their microbial leakage are very limited, and there are no study that evaluated it under functional loading.

In a study by Nassar and Abdalla,[7] evaluated microbial microleakage at two types of connection geometries in an implant system with slip-joint design at its interface. They concluded that the implants with internal hexagon connection had more microleakage than implants with trilobe connection under static condition. In another study Khajavi, *et al*.[10] compared the rate of microleakage of two implant systems with slip joint and conical interface designs using outward method, under static condition. They reported that bacterial leakage in implants with conical connection was significantly more than slip joint design, but no significant difference was seen over time. In comparison to our study, the results of microbial leakage in implants with slip joint connection under static condition is almost the same (in 10% of samples after 5 days vs. in 20% after 2–7 days). However, there are significant difference in the result of colony counts in the same groups between the two studies (4×10^3 CFU/mL vs. 4×10^5 CFU/mL), which may relate to the method of experiment. The present study showed lower colony counts in the same group using inward method, which are more similar to the oral environment.

Since, there is no study that evaluated the effect of dynamic loading on the microbial leakage of implants with slip joint connection, we evaluated this factor in the present study to compare with the results of microleakage at static condition.

There are different *in vitro* studies which evaluated the influence of cyclic loading on the microbial leakage of implants in a unique interface design or among several connection geometries and mostly founded the same results to the present study. In the present study, the results of the relative frequency distribution of microbial leakage from the implant-abutment connection indicated more samples with microleakage in the loaded group than unloaded (50% versus 10%), and more contamination with higher colony counts (27,000 CFU/mL vs. 4000 CFU/mL), showing that dynamic loading can increase the rate of microbial leakage at the implant-abutment interface with slip joint design.

In a study by Koutouzis *et al*., to investigate the effect of dynamic loading on the bacterial leakage at the implant-abutment connection with conical design, using the inward method, reported higher bacterial leakage and

colonization after loading, similar to the results of the present study.[1] Whereas, Tripodi *et al.* in their outward evaluation of microbial microleakage at the interface of an implant system with conical design, founded low rate of microleakage with no significant difference between groups with/without dynamic loading. This inconsistency with our study can be related to the difference in factors such as type of implant system, connection, method of experiment, and time period.[11]

In another study by do Nascimento *et al.* evaluated the saliva leakage at the implant-abutment interface of an implant system with but joint and conical designs in three types of connection, under static and dynamic conditions. The results showed that in all three connections, saliva leakage under loading was significantly more than unloading condition which the results are consistent with the present study.[3]

Due to a few information about the microleakage rate of implants with slip-joint design, it is suggested to compare this kind of interface with another designs like, butt joint or conical under dynamic loading in future studies.

CONCLUSIONS

Microbial microleakage at the implant-abutment interface with slipjoint design increased significantly after functional loading.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Koutouzis T, Mesia R, Calderon N, Wong F, Wallet S. The effect of dynamic loading on bacterial colonization of the dental implant fixture-abutment interface: An *in vitro* study. J Oral Implantol 2014;40:432-7.
2. Siadat H, Arshad M, Mahgoli HA, Fallahi B. Microleakage evaluation at implant-abutment interface using radiotracer technique. J Dent (Tehran) 2016;13:176-83.
3. do Nascimento C, Miani PK, Pedrazzi V, Gonçalves 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;27:551-60.
4. Salihoglu U, Boynuegri D, Engin D, Duman AN, Gokalp P, Balos K. Bacterial adhesion and colonization differences between zirconium oxide and titanium alloys: An *in vivo* human study. Int J Oral Maxillofac Implants 2011;26:101-7.
5. Bishti S, Strub JR, Att W. Effect of the implant-abutment interface on peri-implant tissues: A systematic review. Acta Odontol Scand 2014;72:13-25.
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;4:158-61.
7. Nassar HI, Abdalla MF. Bacterial leakage of different internal implant/abutment connection. Futur Dent J 2015;1:1-5.
8. Misch CE. Dental Implant Prosthetics. 2nd ed. United State: Mosby, Elsevier Inc.; 2014.
9. Romanos GE, Biltucci MT, Kokaras A, Paster BJ. Bacterial composition at the implant-abutment connection under loading *in vivo*. Clin Implant Dent Relat Res 2016;18:138-45.
10. Khajavi A, Mohseni S, Peymani A, Amjadi M. *In vitro* bacterial leakage at the implant-abutment connection of two dental implant systems with internal connection. Front Dent 2020;17:32.
11. Tripodi D, D'Ercole S, Iaculli F, Piattelli A, Perrotti V, Iezzi G. Degree of bacterial microleakage at the implant-abutment junction in cone Morse tapered implants under loaded and unloaded conditions. J Appl Biomater Funct Mater 2015;13:e367-71.
12. Harder S, Podschun R, Grancicova L, Mehl C, Kern M. Analysis of the intraimplant microflora of two-piece dental implants. Clin Oral Investig 2013;17:1135-42.
13. 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;31:269-75.
14. da Silva-Neto JP, Nóbilo MA, Penatti MP, Simamoto PC Jr., 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;27:793-800.
15. de Oliveira GR, Olate S, Pozzer L, Cavalieri-Pereira L, Rodrigues-Chessa JG, Albergaría-Barbosa JR. Bacterial contamination along implant-abutment interface in external and internal-hex dental implants. Int J Clin Exp Med 2014;7:580-5.
16. Gherlone EF, Capparé P, Pasciuta R, Grusovin MG, Mancini N, Burioni R. Evaluation of resistance against bacterial microleakage of a new conical implant-abutment connection versus conventional connections: An *in vitro* study. New Microbiol 2016;39:49-56.
17. 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;21:123-9.
18. 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;20:732-7.
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;14:654-60.
20. 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;72:1146-50.
21. Tesmer M, Wallet S, Koutouzis T, Lundgren T. Bacterial colonization of the dental implant fixture-abutment interface: An *in vitro* study. J Periodontol 2009;80:1991-7.
22. Silva-Neto JP, Prudente MS, Carneiro Tde A, Nóbilo MA, Penatti MP, Neves FD. Micro-leakage at the implant-abutment interface with different tightening torques *in vitro*. J Appl Oral Sci 2012;20:581-7.
23. Harder S, Dimaczek B, Açil 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;14:427-32.
24. Jansen VK, Conrads G, Richter EJ. Microbial leakage and marginal fit of the implant-abutment interface. Int J Oral Maxillofac Implants 1997;12:527-40.
25. Gil FJ, Herrero-Climent M, Lázaro P, Rios JV. Implant-abutment connections: Influence of the design on the microgap and their fatigue and fracture behavior of dental implants. J Mater Sci Mater Med 2014;25:1825-30.