

Comparative Analysis of Microbial Leakage in Implant Recess of Three Different Internal Implant Abutment Connections: An *In Vitro* Study

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ABSTRACT

Objective/Introduction: Bacterial infiltration at implant abutment junction is a potential risk for soft tissue inflammation and bone loss around the implant. Potential colonization of microgap is related to precision of fit at the mating interfaces of implant and abutment and is dependent on structural geometry of implant abutment connection. Considering the limited information regarding differences in microbial penetration at the junction in implants with degree of taper in internal connections, this study conducted comparative analysis of microbial seal of three different implant abutment connections—1.5 mm internal hex (IH), 4° tapered, and 11° conical hex (CH) implants.

Materials and methods: Three study groups with 18 implant abutment assemblies in each, included 1.5 mm IH (BioHorizon®), 4° taper (4° CH) internal conical connection (Ankylos®), and 11° taper (11°CH) connection (Neobiotech® Active) implants. Each assembly was suspended in *Escherichia coli* as well as *Enterococcus faecalis* suspension in brain heart infusion (BHI) separately and incubated at 37°C for 3 days. Wash from respective implant internal recess was assessed for microbial contamination.

Results: Internal hex group showed significantly higher *E. coli* (44.4%) and *E. faecalis* (50.0%) leakage than 4°CH and 11°CH groups. The difference in microbial leakage in 4°CH (11.1%) and 11°CH (16.7%) for both the study microbes was statistically insignificant ($p = 0.630$), thus partially accepting the null hypothesis.

Conclusion: The type of connection significantly influences microbial leakage at implant abutment junction with internal connection more conducive to bacterial infiltrations than conical connection implants. However, degree of taper of conical connection does not have a significant effect on bacterial permeability of the implant abutment connection.

Keywords: Bacterial infiltration, Dental implants, Implant abutment connection, Implant recess, Internal hex, Microbial leakage.

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INTRODUCTION

Implants are commonly used in the treatment of partially and completely edentulous patients. The implant–abutment interface (IAI) is the connection between the implant and abutment which can be either single piece or a two-part system.

In a two-part implant abutment system, a microgap is present at IAI, through which the seepage of fluid and micro molecules from the saliva, crevicular fluid, and bacteria occurs during cyclic loading of the implant supported crown.¹ The presence of bacteria in the internal recess of implant and penetration of the bacteria through microgap may increase the risk of soft tissue inflammation and bone loss around the implant.² Potential colonization of microgap is probably related to precision of fit between implant components and loading forces when implants are in function.³

According to Binon et al., the microgap between IAI varies between different implant systems and reported to be 20–50 µm.⁴ Jansen et al. found this microgap to be 5 µm, whereas according to Callan et al. microgap varies between 30 and 135 µm.^{5,6} The type of implant–abutment connection (IAC) plays significant role in bacterial leakage and the subsequent peri-implant inflammation. External hex and internal hex (IH) connections are slip-fit joints with a microgap of 20–25 µm, whereas conical tapered connections are a tube in tube friction fit connections with much narrower gap of 2–3 µm and a cold welding which delays and even prevents bacterial infiltration.^{4,7} Microgap in conical connection implants depends on degree of taper of the mating components which

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varies from 4 to 11 degrees in most conical connection implants. Various studies have reported higher degree of bacterial infiltration in IH connection implants as compared to conical connection implants.^{1,8–10} Schmitt et al. in their systematic review reported conical connection implants to have lesser microgap and better stability and microbial seal than butt joint.¹¹

There is limited information regarding differences in microbial penetration of the microgap of implants with different degree of taper in friction fit connection implants. This *in vitro* study aims to comparatively evaluate the sealing ability of three different

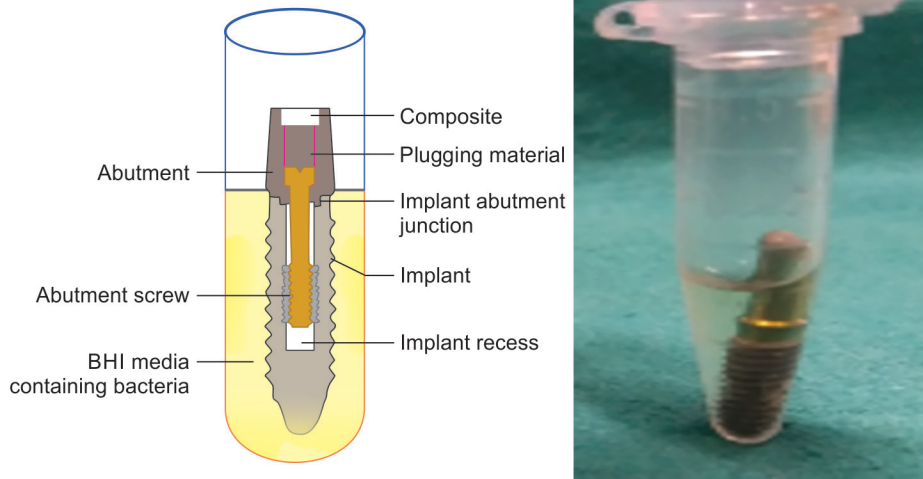


Fig. 1: Implant abutment complex in vertical position

IAC—1.5 mm IH, 4° tapered and 11° conical hex (CH) implant. Null hypothesis was that there is no difference in microbial permeability of IH connections and conical connection implants with different degree of taper.

MATERIALS AND METHODS

A total of 54 implant abutment assemblies with three different implant connections (*n* = 18 in each subgroup) were used in this study.

Preparation of Implant Abutment Complexes

For each group, 18 sterile implants (BioHorizon®, Ankylos® C/by Dentsply Sirona, IS-II Active Neobiotech®) with 1.5 mm IH group, 4° tapered (4° CH) group, and 11°CH group connection, respectively, were taken and the respective abutments were screw tightened using 30, 15, and 30 Ncm torque as per respective manufacturer recommendation using the cordless auto torque driver (MEG TorQ by Megagen® South Korea).

Preparation of Samples for Microbiological Analysis

The implant abutment complexes were placed in vertical position in the Eppendorf tube and *Escherichia coli* bacterial suspension in brain heart infusion (BHI) was added in Eppendorf tube with micropipette such that implant abutment junction was dipped (Fig. 1). Each implant in Eppendorf tube with bacterial suspension was incubated at 37°C for 30 days. The bacterial suspension (*E. coli*) was replaced every 4th day to prevent the overgrowth of the bacteria in the media. After 30 days of above procedure, the composite sealing the screw access hole and plugging material was removed.

Sample from Implant Internal Recess

Abutments were unscrewed carefully and implant fixture recess was rinsed with premeasured quantity of saline in a sterile syringe. The saline was immediately drawn up and transferred into a sterile Eppendorf tube having peptone water. The wash from implant recess was then planted on MacConkey Agar for microbial analysis.

Same procedure as above was followed for implant abutments assemblies of all the groups using *Enterococcus faecalis* suspension in vertical position of the assemblies in the Eppendorf tube.

Table 1: Microbial analysis of wash obtained from the internal recess of three study groups

	Presence of bacteria in implant recess			
	Presence of <i>E. coli</i>		Presence of <i>E. faecalis</i>	
	Absent	Present	Absent	Present
Connections				
IH				
Count	10	8	9	9
% within connection	55.6	44.4	50.0	50.0
4° CH				
Count	16	2	16	2
% within connection	88.9	11.1	88.9	11.1
11° CH				
Count	15	3	15	3
% within connection	83.3	16.7	83.3	16.7
<i>p</i> -value	0.043		0.016	

Table 2: Chi-square test values for intergroup comparison

	<i>p</i> -value		
	<i>E. coli</i>	<i>E. faecalis</i>	
IH vs 4° CH	0.026	0.011	Significant
IH vs 11° CH	0.070	0.034	Significant
4° CH vs 11° CH	0.630	0.630	Non-significant

RESULTS

Microbial contamination of implant recess in each assembly was assessed and the data collection was analyzed statistically using Chi-square test.

Microbial Analyses of Wash from 3 IAC

Internal hex connection implants (Group IH) showed significantly higher *E. coli* (44.4%) and *E. faecalis* (50.0%) infiltration than 4°CH and 11°CH connection implants (Tables 1 and 2).

DISCUSSION

The present study confirms low bacterial permeability of conical hex IAC with 4° as well 11° taper and high prevalence of bacterial penetration of internal slip fit connection implants, thus rejecting the first part of the null hypothesis. However, microbial leakage difference between 4° and 11° tapered conical connection implants was found statistically insignificant ($p > 0.05$). Potential colonization of microgap at implant abutment junction (IAJ) is related to IAC type, precision of fit and connecting torque.¹⁰ Loading forces further increase the microgap but their influence is not uniform in all connections and host sites. Internal conical connection implants are reportedly more stable with a tighter connections than butt joint connections like IH.^{9,10,12–14}

Lesser taper in Morse connection creates better seal due to friction lock between the mating components.¹⁵ Microbial ingress of internal conical connection with varying degree of taper (4° and 11°) has been evaluated in present study over an observation period of 30 days. Several studies have depicted two-way microbial leakage into and out of implant abutment internal recess in *in vitro* setups.^{5,9,13,14,16–18} Inward microbial ingress method simulating *in vivo* condition was used in the present study.

Gram-negative anaerobic bacteria have been found surrounding the failing implants in higher counts as compared to healthy implant sites.^{19,20} *Escherichia coli*, a Gram-negative facultative anaerobic rod with 1–2 µm length and 0.5 µm radius, was chosen as contaminating bacteria for this study because of its motility, easy handling, and its short generation time of 20 minutes. Also, it has been isolated from periimplantitis lesions.⁹ Another non-motile Gram-positive facultative anaerobe, *E. faecalis* with 0.5–1 µm dimensions was also used to check the bacterial ingress into IAC due to small size and non-motile nature. Morse taper implant connections have a much lesser microgap of 2–3 µm than 10 µm of slip fit connections.²¹ This fact was taken into consideration while choosing organism of <2 µm so that bacteria could penetrate even the supposedly tight-fitting conical connections.

Previous studies investigated microbial leakage over a period of 14 days which was reported sufficient for leakages through IAC by Aloise et al.⁹ However, Tripodi et al. in 2012 showed that microleakage occurred after 22 days in Morse taper connection so microbial leakage in the present study was evaluated over observation period of 30 days.²²

Cyclic loading in clinical set ups differentially influences degree of micro leakage and further increase the number and type of bacteria.²³ The present study aimed to establish differential microbial leakage in three IACs and required consistent conditions. Hence, cyclic loading was excluded from the study protocol.

The present study reported significantly higher microbial leakage in IH group than 4° and 11° conical connection implants ($p < 0.05$). These results are supported by previous studies by Tripodi et al. and D'Ercole S, who had reported that conical connection implants depicted less microbial leakage than IH connection implants.^{22,24–26} Tesmer et al. reported that only 30% of internal conical connection implants showed microleakage as compared to 90–100% microleakage in trilobed connection.¹⁰ Khajavi A in 2020, reported higher microleakage in Argon conical connection implant as compared to Zimmer slip fit IH connection.²⁷ One degree taper in IH of Zimmer implant abutment surfaces imparts them with high degree of friction lock seal that accounts for better microbial seal.

Our study establishes limiting effect of internal conical connection on microbial leakage at IAJ with both 4° and 11° taper implants effecting similar microbial seal. Aloise et al.,⁹ Deconto et al.,²⁸ and Alves et al.²⁹ had also reported insignificant difference in microleakage in different conical connection implants. The present study confirmed equally low permeability of conical connections with different taper between mating components over slip fit hexagonal connections over a period of 30 days.

CONCLUSION

The type of connection significantly influences microbial leakage at implant abutment junction with internal connection more conducive to bacterial infiltrations than conical connection implants. However, degree of taper of conical connection does not have a significant effect on bacterial permeability of the IAC.

Further research is required to validate the result of this study by increasing the sample size and cyclic loading of the samples. Similar *in vivo* studies need to be conducted to correlate the results in laboratory setups with clinical results.

REFERENCES

1. Amjadi M, Khajavi A, Youssefi N, et al. 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(4):420. DOI: 10.4103/jips.jips_96_21.
2. Ericsson I, Persson LG, Berglundh T, et al. Different types of inflammatory reactions in peri-implant soft tissues. *J Clin Periodontol* 1995;22:255–261. DOI: 10.1111/j.1600-051x.1995.tb00143.x.
3. Piattelli A, Scarano A, Paolantonio M, et al. Fluids and microbial penetration in the internal part of cement-retained versus screw-retained implant-abutment connections. *J Periodontol* 2001;72:1146–1150. DOI: 10.1902/jop.2000.72.9.1146.
4. Binon P, Weir D, Watanabe L, et al. In Laney WR: *Implant component compatibility. Tissue Integration in Oral, Orthopaedic and Maxillofacial Reconstruction*. Chicago: Quintessence; 1993, pp. 218–226.
5. Jansen VK, Conrads G, Richter EJ. Microbial leakage and marginal fit of the implant-abutment interface. *Int J Oral Maxillofac Implant* 1997;12(4):527–540.
6. Callan DP, O'Mahony A, Cobb CM. Loss of crestal bone around dental implants: A retrospective study. *Implant Dent* 1998;7:258–266. DOI: 10.1097/00008505-199807040-00003.
7. Larrucea V, Jaramillo N, Acevedo A, et al. Microleakage of the prosthetic abutment/implant interface with internal and external connection: In vitro study. *Clin Oral Impl Res* 2014;25:1078–1083. DOI: 10.1111/clr.12217.
8. 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.
9. Aloise JP, Curcio R, Laporta MZ, et al. Microbial leakage through the implant-abutment interface of Morse taper implants in vitro. *Clin Oral Implant Res* 2010;21:328–335. DOI: 10.1111/j.1600-0501.2009.01837.x.
10. 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. DOI: 10.1902/jop.2009.090178.
11. Schmitt CM, Nogueira-Filho G, Tenenbaum HC, et al. Performance of conical abutment (Morse Taper) connection implants: A systematic review. *J Biomed Material Res A* 2014;102:552–574. DOI: 10.1002/jbm.a.34709.
12. Romanos GE, Biltucci MT, Kokaras A, et al. Bacterial composition at the implant-abutment connection under loading in vivo. *Clin Implant Dentistry Related Res* 2016;18:138–145. DOI: 10.1111/cid.12270.

13. Dibart S, Warbington M, Su MF, et al. In vitro evaluation of the implant-abutment bacterial seal: The locking taper system. *Inter J Oral Maxillofac Implants* 2005;1:20.
14. Koutouzis T, Wallet S, Calderon N, et al. Bacterial colonization of the implant-abutment interface using an in vitro dynamic loading model. *J Periodontol* 2011;82:613–618. DOI: 10.1902/jop.2010.100415.
15. Macedo JP, Pereira J, Vahey BR, et al. Morse taper dental implants and platform switching: The new paradigm in oral implantology. *Eur J Dent* 2016;10:148–154. DOI: 10.4103/1305-7456.175677.
16. 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(6): 875–881.
17. Persson LG, Lekholm U, Leonhardt Å, et al. Bacterial colonization on internal surfaces of Brånemark system® implant components. *Clin Oral Implants Res* 1996;7(2):90–95. DOI: 10.1034/j.1600-0501.1996.070201.x.
18. Coelho PG, Sudack P, Suzuki M, et al. In vitro evaluation of the implant abutment connection sealing capability of different implant systems. *J Oral Rehabilitation* 2008;35(12):917–924. DOI: 10.1111/j.1365-2842.2008.01886.x.
19. Augthun M, Conrads G. Microbial findings of deep peri-implant bone defects. *Int J Oral Maxillofac implants* 1997;12(1):106–112.
20. Salcetti JM, Moriarty JD, Cooper LF, et al. The clinical, microbial, and host response characteristics of the failing implant. *Int J Oral Maxillofac Implants* 1997;12(1):32–42.
21. Larrucea Verdugo C, Jaramillo Núñez G, Acevedo Avila A, et al. Microleakage of the prosthetic abutment/implant interface with internal and external connection: In vitro study. *Clin Oral Implants Res* 2014;25:1078–1083. DOI: 10.1111/clr.12217.
22. Tripodi D, Vantaggiato G, Scarano A, et al. An in vitro investigation concerning the bacterial leakage at implants with internal hexagon and Morse taper implant-abutment connections. *Implant Dent* 2012;21:335–339. DOI: 10.1097/ID.0b013e31825cd472.
23. Raab P, Alamanos C, Hahnel S, et al. Dental materials and their performance for the management of screw access channels in implant-supported restorations. *Dent Mater J* 2017;36(2):123–128. DOI: 10.4012/dmj.2016-049.
24. 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;7:115–120. DOI: 10.1902/jop.2005.76.1.115.
25. D’Ercole S, Scarano A, Perrotti V, et al. Implants with internal hexagon and conical implant-abutment connections: An in vitro study of the bacterial contamination. *J Oral Implantol* 2014;40(1):30–34. DOI: 10.1563/AAID-JOI-D-11-00121.
26. Gherlone EF, Capparé P, Pasciuta R, et al. Evaluation of resistance against bacterial microleakage of a new conical implant-abutment connection versus conventional connections: An in vitro study. *New Microbiol* 2016;39(1):49–56.
27. Khajavi A, Mohseni S, Peymani A, et al. In vitro bacterial leakage at the implant-abutment connection of two dental implant systems with internal connection. *Front Dent* 2020;17:32. DOI: 10.18502/vid.v17i32.5196.
28. Deconto MA, Salvoni AD, Wassall T. In vitro microbiological bacterial seal analysis of the implant/abutment connection in morse taper implants: A comparative study between 2 abutments. *Implant Dent* 2010;19(2):158–166. DOI: 10.1097/ID.0b013e3181cc48b1.
29. Alves DC, Carvalho PS, Martinez EF. In vitro microbiological analysis of bacterial seal at the implant-abutment interface using two morse taper implant models. *Braz Dent J* 2014;25:48–53. DOI: 10.1590/0103-6440201302178.