ORIGINAL RESEARCH

Evaluation of Microleakage and Microgap of Two Different Internal Implant–Abutment Connections: An *In Vitro* Study

Vulli VS Triveni[1], Kyatsandra N Jagadeesh[2], Anuj Singh Parihar[3], Sami Alduwayhi[4], Sridhar Annapoorneshwari[5], Faiz Muslimveetil Khalid[6], Prashant Babaji[7]

Abstract

Aim: The higher success rate (>90%) of dental implants over 5 years has made this treatment option favorable for dental surgeons as well as for patients. The present *in vitro* study was conducted to assess microleakage and microgap of two dissimilar internal implant–abutment associations.

Materials and methods: Forty dental implants were divided into two groups: trilobe internal connection fixtures in group I and internal hexagonal geometry fixtures in group II. For the immersion of implant abutment assemblies, sterilized tubes containing 4 mL of *Staphylococcus aureus* broth culture were incubated at 37°C for 2 weeks. Gram's stain and biochemical reactions were used for identification of colonies.

Results: The mean $\log_{10}$ colony-forming unit (CFU) in group I was 8.6 and was 9.3 in group II. The disparity among two groups was found to be significant ($p < 0.05$). The mean microgap in group I was 7.2 µm and was 10.4 µm in group II. The disparity among the two groups was found to be significant ($p < 0.05$).

Conclusion: Authors found that microscopic space between implant and abutment may be the site of penetration of bacteria. There was significant higher $\log_{10}$ CFU in dental implant fixtures with an internal hexagonal geometry compared to the dental implant fixtures with a trilobe internal connection.

Clinical significance: Microscopic space between implant and abutment may be the site of penetration of bacteria. This information will help to avoid microleakage to improve implant success rate.

Keywords: Bacteria, Dental implants, *Staphylococcus aureus*.

The Journal of Contemporary Dental Practice (2020): 10.5005/jp-journals-10024-2817

Introduction

With the advent of dental implants, dentistry gained its importance. The higher success rate of >90% over 5 years has made this treatment option favorable for dental surgeons as well as for patients.[1] However, in spite of the exceptional success rates in osseointegration of dental implants, many shortcomings have been mentioned regarding surgical techniques and mechanical microbiological factors. Peri-implantitis is a recent and the most commonly occurring pathology of the dental implant. Bacteria and their products may cause inflammatory reactions in soft tissues around the dental implants. This has opened the eyes to look for various pathologic bacteria responsible for treatment failure.[2]

Gingival recession may be the result of peri-implant bone loss. There is subsequent recession in the height of the papilla owing to the increased distance between the contact point of the teeth and the crest of alveolar bone.[3] Recent studies mentioned the role of microbial leakage at the implant–abutment connection, which is the main hindrance in the construction of the two-stage implant systems.[4] These microbial leakages are due to breach and opening which are formed among the implant and the abutment. This in turn initiates peri-implant inflammatory reactions. It has been observed that fit precision among the fixture and abutment and tightening torque and micro movements among the connected apparatus during mastication determine the amount of bacterial colonization between the implants and abutments.[5] The present *in vitro* study was conducted to assess microleakage and microgap of two dissimilar internal implant abutment association: trilobe connection and internal hexagonal connection.

[1]Department of Dentistry (Prosthodontics), Rangaraya Medical College, Kakinada, Andhra Pradesh, India

[2]Department of Prosthodontics and Implantology, Sree Siddharth Dental College, Sree Siddharth Academy of Higher Education, Tumkur, Karnataka, India

[3]Department of Periodontics, Peoples Dental Academy, Bhopal, Madhya Pradesh, India

[4]Department of Prosthodontics, College of Dentistry, Majmaah University, Al-Majmaah, Kingdom of Saudi Arabia

[5]Private Practitioner, Bengaluru, Karnataka, India

[6]Department of Prosthodontics, PSM College of Dental Sciences and Research, Akkikkavu, Thrissur, Kerala, India

[7]Department of Pediatric and Preventive Dentistry, Vyas Dental College, Jodhpur, Rajasthan, India

Corresponding Author: Vulli VS Triveni, Department of Dentistry (Prosthodontics), Rangaraya Medical College, Kakinada, Andhra Pradesh, India, Phone: +91 8827047003, e-mail: dr.anujparihar@gmail.com

How to cite this article: Triveni VVS, Jagadeesh KN, Parihar AS, *et al.* Evaluation of Microleakage and Microgap of Two Different Internal Implant–Abutment Connections: An *In Vitro* Study. J Contemp Dent Pract 2020;21(6):683–685.

Source of support: Nil

Conflict of interest: None

Materials and Methods

The present *in vitro* study was conducted in the Department of Prosthodontics and Implantology, India. Approval of the study was

obtained from ethics committee of institute, Peoples Dental Academy, Bhopal. It comprised 40 dental implants (3.0 mm × 10 mm; Biohorizon), which were divided into two groups with 20 samples each. In group I, the fixtures with a trilobe internal association were linked to 0.3 cm high abutments of 35 Ncm torque and in group II fittings with an internal hexagonal geometry were associated with typical straight abutments with a depth of 0.6 cm and a torque of 25 Ncm.

In present study, *Staphylococcus aureus* was used. A bacterial suspension was prepared in brain–heart infusion (BHI) broth by cultivating *S. aureus* and incubating it at 37°C for 24 hours. This suspension was diluted in nutrient broth to attain a compactness of 1×10^8 CFU per mL.

Microbial Sampling

Dental implants were detached and held in vertical position to allow firm torque action under strict sterilized conditions. Abutments were then attached with dental implants. Tubes having sterile BHI broth were used for the immersion of implant–abutment assembly for 30 seconds. The tubes were then incubated at 37°C for 2 weeks.

Following this, the implant–abutment assembly was sunken in test tubes having 4 mL of *S. aureus* broth culture and was incubated at 37°C for 2 weeks. After 2 weeks of immersion, the assembly was removed and held in 70% alcohol for 3 minutes. After this, bacterial contamination was done using sterile paper points along the inner surfaces of the implants. Sterile paper points were inoculated in test tubes having sterile BHI broth. Culture was done on blood agar plates from broth and incubated at 37°C for 24 hours. The resultant colonies were noted using Gram's stain and biochemical response.

Microgap Assessment

The microgap was evaluated in all samples using an electron microscope at a voltage of 15 kV. The extent of the microgap was evaluated and measured at four points for each samples.

Results thus obtained were entered in Excel sheets. The SPSS Statistical software version 21.0 was used. A comparison between groups was done with Mann–Whitney *U* test and the microgap was evaluated using Turkey's honestly significant difference (HSD) test.

Results

Table 1 shows two types of implant systems: in group I, fixtures were trilobe connection and in group II fixtures were of internal hexagonal connection. Each group comprised 20 dental implants. Table 2 shows that mean $\log_{10}$ CFU, which was 8.6 in group I and 9.3 in group II. The distinction between the two assemblies was found to be considerable ($p < 0.05$). The mean microgap in group I was 7.2 µm and in group II 10.4 µm. The dissimilarity among the two groups was found to be considerable ($p < 0.05$) (Tables 3 and 4). Lower microleakage and microgap were observed; hence, lower microbial CFU in group I as compared to group II.

Table 1: Distribution of dental implants

Groups	*Group I*	*Group II*
Fixtures	Trilobe connection	Internal hexagonal connection
Number	20	20

Table 2: Comparison between $\log_{10}$ colony-forming unit in the both groups

Groups	*Mean*	*SD*	*Median*	*p value*
Group I	8.6	0.2	8.4–8.8	0.05
Group II	9.3	0.4	9.1–9.5	

Discussion

The microscopic space between implant and abutment can act as a bacterial niche, favoring the loss of the peri-implant mucosal seal.[6,7] These microgaps may alter the clinical and microbiological parameters of tissues with increase in bacterial load precipitating periodontal diseases, which in turn lead to failure in osseointegration. It has been further postulated that these spaces favor penetration of fluids and saliva, leading to bacterial invasion.[8,9]

The gap sizes may alter bacterial contamination. Certain factors such as torque, precision of fit, and micromovement affects the level of contamination. Broggini et al.[10] found infiltration of white blood cells around the implants, which vary according to the implant design.

The present *in vitro* study was conducted to assess bacterial seepage of two unlike internal implant abutment associates. In the present study, 40 dental implants (3.0 mm × 10 mm; Biohorizon) were divided into two groups of 20 samples each. Group I equipped with a trilobe internal relationship was associated with 0.3 cm elevated abutments; and group II equipped with an internal hexagonal geometry was linked to the standard straight abutments with a height of 0.6 cm.

In present study, we used only internal hexagonal and trilobe connection because both demonstrated different types of connection, thereby showing different response to dynamic loading.

Saidin et al. stated from their study that internal conical abutment resulted in higher degree of micromovement, whereas the trilobe association resulted in the lower degree of micromovement due to its polygonal profile.[11] Hence, lower microleakage and microgap was observed with trilobe connection. Therefore, in the present study, group I (trilobe connection) showed lower microleakage and bacterial content than group II (connection internal hexagonal connection).

Faria et al. conducted an *in vitro* study and assessed bacterial seepage beside the implant–abutment border, comparing three types of associates: external hexagon (EH), Morse taper (MT), and internal hexagon (IH).[12] In this study, authors used apical portion of abutment screws for the inoculation of colonies of *Escherichia coli*, which later on were fixed to implants. Samples that had exterior contamination were excluded, whereas residual specimens were positioned in test tubes enclosing tryptic soy broth. In this study, 38 samples with external hexagonal, 40 with internal hexagonal, and 41 with Morse taper connections were determined. The value for external hexagonal was 10.53%, for internal hexagonal was 4.88%, and for Morse taper was 7.50% connections. There were no differences between all connections ($p > 0.05$).

In present study, the mean $\log_{10}$ CFU in group I was 9.3 and in group II 8.6. The disparity among both the groups was found to be significant ($p < 0.05$). Nassar et al. conducted a study to estimate the bacterial seepage of two dissimilar internal implant abutment

Table 3: Assessment of microgap

Groups	*Mean (µm)*	*SD*	*Min.*	*Max.*
Group I	7.2	5.4	2.1	13.4
Group II	10.4	3.8	4.3	16.1

Table 4: Two-by-two comparisons of mean microgap by Turkey's honestly significant difference test

Groups	*Subset for $\alpha = 0.05$*	*Significant*
Group I	7.2	0.01
Group II	10.4	

associations. They divided dental implants into two groups with 10 dental implants. Authors found significant higher mean $\log_{10}$ CFU with internal hexagon implants as compared to trilobe implants.[13] These findings are in association with our result.

We found that the mean microgap was 7.2 µm in group I and 10.4 µm in group II. The disparity among two groups was found to be significant ($p < 0.05$).

Tesmer et al. conducted a study to determine bacterial migration at the interface of dental implant fixture and abutment. Thirty implants were categorized into three groups depending on their microgap. Groups I and II consist of fixtures with internal Morse taper associates and in group III consists of trichannel internal connection in dental implants. Fixtures and abutments were subjected to bacterial concentration of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. Significantly lower bacterial colonization was observed in group I compared to groups II and III. They concluded that variation in implant designs could influence the bacterial seepage.[14]

Koutouzis et al. evaluated the outcome of dynamic loading on the microbial colonization in the fixture-abutment interface (FAI) microgap of dental implants with internal Morse taper association. They concluded that implants with internal Morse taper association display least bacterial incursion and bacterial infiltration increases with dynamic loading.[15]

Wachtel et al. evaluated the bacterial leakage of the implant-abutment interface (IAI) of two-piece implant systems by using suspension of *Enterococcus faecium* before abutment fixation. There was bacterial leakage before the cyclic loading in three of the seven implants.[16]

Rismanchian et al. evaluated microgap extent and microbial seepage in the association area of four dissimilar abutments to International Team for Implantology (ITI) implants. Authors found significant mean microgap size in different types of abutments and found average number of seep out colonies through the association of the implant and abutment surface.[17]

A broad range of microorganisms found to be penetrating along the implant abutment interface such as gram-positive cocci to gram-negative rods (*Bacteroides* species, *Fusobacterium* species, and *Peptostreptococcus micros*), which are connected with peri-implantitis. Various studies have shown that microleakage at implant abutment interface can be evaluated using various microorganism such as *A. actinomycetemcomitans*, *P. gingivalis*, *E. coli*, *Ent. faecium*, or *S. aureus*.[13–16,18] In the present study, we used *S. aureus* to evaluate the microleakage.

Present implant systems may not completely avoid microbial seepage and bacterial migration of the inner part of the implant. The diffusion of oral microorganisms through the implant abutment border may create soft tissue inflammation and affects the clinical success of implants.[18]

The present study indicates that microgap acts as the site for penetration of bacteria leading to failure of implant. This microgap should be avoided to improve the implant success rate. Further studies are required to evaluate the microgap and microleakage using different types of implant assembly with dynamic loading.

Conclusion

Authors found that microscopic space between implant and abutment may be the site of penetration of bacteria. Significantly higher $\log_{10}$ CFU was observed in dental implant fixtures with an internal hexagonal geometry compared to dental implant fixtures with a trilobe internal connection.

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