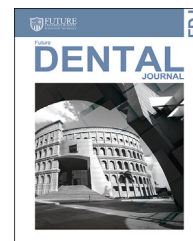


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Bacterial leakage of different internal implant/abutment connection

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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 Log₁₀ 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.

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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

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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 $4.0 \times 12 \text{ mm}$) (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.

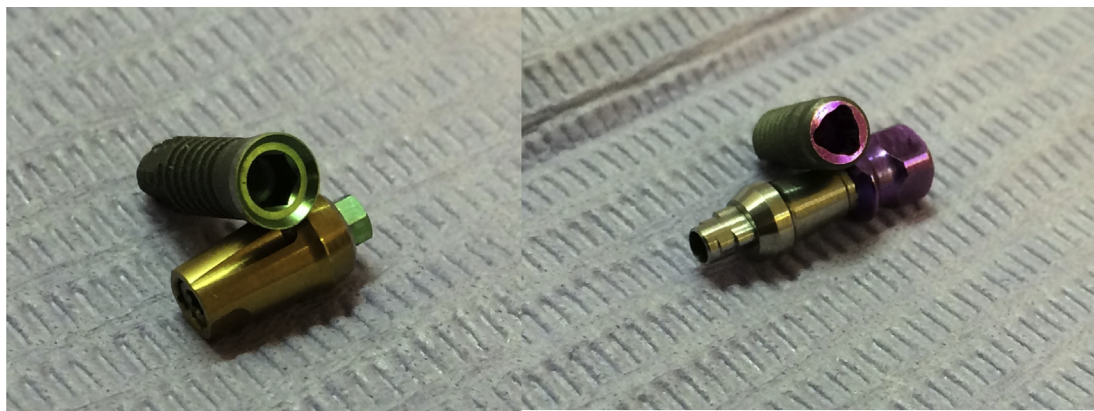


Fig. 1 – Trilobe connection and internal hexagon connection respectively.



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 Log₁₀ 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 ≤ 0.05.			

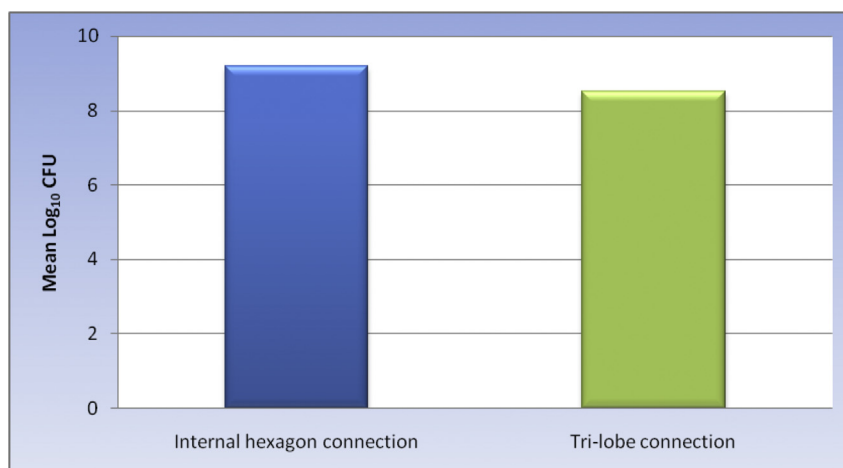


Fig. 3 – Bar chart representing mean Log₁₀ CFU in the two groups.

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 (Nobel-pharma 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 peri-implantitis [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.

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