



Assessment of Bacterial Leakage at the Implant-Abutment Interface of Internal and External Connection Implants: An *In Vitro* Study

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Abstract

Objective: The presence of misfit at the implant-abutment interface can cause the accumulation of a bacterial biofilm, which leads to peri-implant bone loss, thereby compromising the long-term outcome of osseointegrated implants. The aim of this *in vitro* study was to assess bacterial leakage in external and internal connection implants.

Methods: Twenty-four samples were analyzed, including 12 external connection implants (Group 1) and 12 internal connection implants (Group 2). In order to assess bacterial leakage, 0.3 µL of a suspension containing *Escherichia coli* was inoculated in the hollow internal part of the implants. The prosthetic abutments were then installed and provided the torque recommended by the manufacturer. The samples were placed in test tubes containing a brain-heart infusion medium, and bacterial leakage was observed at 24, 48, and 72 hours, and at 7 and 14 days.

Results: No leakage was observed in Group 1 throughout the study period, whereas Group 2 showed leakage in 1 sample.

Conclusion: The low amount of bacterial leakage observed at the implant-abutment interface of these samples highlights the appropriate sealing.

Keywords

Bacterial leakage; Prosthetic implant-abutment interface; Osseointegration; Dental implant; *Escherichia coli*; Microgap; Hexternal hex; Internal prosthetic connection

Introduction

Several longitudinal studies have shown the long-term success of osseointegrated implants [1,2]. However, certain factors can have a negative influence on Long time success rate of implants, including the adaptation between the implant and the prosthetic abutment.

The existence of empty spaces at the prosthetic implant-abutment interface (I-A interface) favors the accumulation of a bacterial

biofilm, which can result in inflammation of the peri-implant tissue. *In vitro* [3-6] and *in vivo* [7-10] studies have shown the capacity of bacteria to infiltrate the implant-abutment interface in different prosthetic platforms. The accumulation of a bacterial biofilm at the interface can affect the progress of treatment and interfere with the esthetic and functional long-term success of a prosthetic device. Quirynen et al. [11] observed that a wide range of microorganisms seem to be able to penetrate implant components, from gram-positive cocci to gram-negative rods. The authors found bacteria such as *Streptococcus constellatus*, *Bacterioides* sp., *Peptostreptococcus micros*, and *Fusobacterium* sp. associated with peri-implantitis inside Brånemark System implants. The presence of microorganisms at the I-A interface can lead to the presence of inflammatory infiltrates close to the interface [12,13].

A classic study assessed the interfaces of different implants and their respective prosthetic abutments in 13 systems, applying different models of the prosthetic interface in relation to bacterial leakage and its critical aspects. In all systems, except one, *Escherichia coli* was found in the solution on the first day in at least one of the samples [3].

Considering that the biofilm that accumulates in cases of misfit of the I-A interface can lead to bone loss, it is important to assess the possibility of bacterial leakage at the interfaces. Thus, the aim of this study was to assess the possibility of bacterial leakage at the I-A interface of two implant systems: one external connection and one internal connection.

Material and Methods

Sample selection

In this study, implants and abutments of two different implant models from the same system were used, which are manufactured and commercialized in Brazil. The selected samples were purchased through the representatives of the respective manufacturer (Table 1).

Twelve test specimens of each model were used, each comprising one implant and its respective prosthetic abutment, for a total of 24 test specimens. These samples were divided into two groups, according to the implant model. Group 1 was comprised of implants of model Kort HEX (4.0×7 mm) (Dérig Implantas, Barueri, São Paulo) with an external hexagon prosthetic connection, and Group 2 was comprised of implants of model Bioneck TRI (3.5×13 mm) (Dérig Implantas, Barueri, São Paulo) with an internal tri-channel connection.

Microbiological tests

The assessment of the possibility of bacterial leakage at the I-A interface was performed according to the methodology previously described [4].

Twelve samples from each group were used: 10 samples for testing, one for a positive control, and one for negative control. Implants were provided in their original packaging, previously sterilized by their respective manufacturer, in addition to their respective prosthetic abutments. The prosthetic abutments were not sterilized by the manufacturer; therefore, they were sterilized using an autoclave (Cristófoli Lister 12L, Cristófoli, Paraná, Brazil) for 22 minutes at 121°C with a pressure of 1.0 KGF/cm², 24 hours before the test. The

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Received: March 01, 2016 Accepted: April 25, 2016 Published: May 02, 2016

Table 1: Characterization of the test specimens.

| System | Implant Model | Prosthetic Abutment | Screwing Torque |
|--------|--------------------------|---------------------|-----------------|
| Dérig | KORT HEX 4.0×7 mm | 02.03.04.112 | 32 Ncm |
| Dérig | BIONECK TRI 3.5×13 mm | 02.04.04.112 | 32 Ncm |

full procedure was performed in sterile conditions, in a disinfected laminar flow cabinet. The operator was properly dressed and stayed within a safety zone provided by the flame of a Bunsen burner. The test specimens were inoculated with 0.3 µL of *E. coli* suspension (reference strain ATCC 25922), applied in the hollow part inside the implant (Figure 1), with the help of a 77FAA10 micropipette (Prolab, Santiago, Chile). The bacteria were kept frozen before use. They were then activated by inoculation in a brain-heart infusion medium (BHI, Kasvi, Italy) and maintained in an incubator at 37°C for 24 hours before the inoculation.

Immediately after inoculation, the prosthetic abutments were connected to their respective implants, and then the torque recommended by the manufacturer was applied to the abutment fixation screw (Figure 2), as described in Table 1. In order to apply the torque to the prosthetic abutment fixation screw, a torque driver from the same manufacturer was used, coupled to the respective manual torquemeter.

After applying the torque, a sterile microbrush (KG Sorensen, Cotia, SP, Brazil) dipped in saline solution was used on the implant platform in order to test the possibility of contamination during inoculation or leakage of the suspension when applying the torque to the abutment (Figure 3). A sterile plastic bit was then mounted at the edge of the prosthetic abutments to prevent them from falling into the test tubes. The test specimens and microbrushes were placed in sterile test tubes containing BHI culture medium, covering approximately 1 mm above the I-A surface (Figure 4).

E. coli is a mobile, bacillus-shaped, gram-negative bacterium that is facultative anaerobic, which has a diameter of 1.1–1.5 µm and a length between 2 and 6 µm. It is widely used in microbiology studies regarding sterilization, disinfection, and *in vitro* contamination [3].

A positive control and a negative control assay were performed for each group. In the positive control assay, 0.3 µL of the solution containing *E. coli* was inoculated directly in the hollow part of the implant, and then the implant was immediately placed in a sterile test tube with the culture medium (BHI) without installing the prosthetic abutment. In the negative control assay, the prosthetic I-A set was placed directly in the test tube with the culture medium without inoculation of *E. coli*.

The test tubes containing the inoculated test specimens and the culture medium, as well as microbrushes and positive and negative controls were incubated in a biological incubator (model Q-316M2 - Quimis Aparelhos Científicos Ltd., Diadema, SP) at 37°C. They were checked for bacteria in the culture medium after 24, 48, and 72 hours and at days 5, 7, and 14 after the inoculation by determining the turbidity of the culture medium (positive or negative) (Figure 5).

From each sample that showed positive results for bacterial leakage, a portion of the contaminated culture medium was collected and transplanted to a petri dish containing an agar/BHI medium, in order to confirm the growth of colonies compatible with *E. coli* growth. The Gram staining method was also applied and the results were observed under an optical microscope, in order to confirm the growth of gram-negative bacillus.

Results

No sample in Group 1 showed bacterial leakage or microbrush contamination during the 14 days of observation. In Group 2, one sample (B-9) showed bacterial leakage after 24 hours. Subsequently, none of the other samples showed leakage. The microbrush used for sample B-6 in Group 2 tested positive for contamination in the first 24 hours, but the implant did not show evidence of bacterial leakage until the end of the observation period.

Discussion

The long-term success of osseointegrated implants depends on the integration between the components of the implant system and oral tissues. Bone loss around the implant is expected in the first year [1,14]. Some potential causes to explain the etiology of this bone loss around the implant have been postulated, including surgical trauma, occlusal overload, peri-implantitis, biological width, and the presence of a misfit at the I-A interface [15].

The inflammatory infiltrate present in the connective tissue at the level of the I-A interface found by Brogginini et al. [13] suggested the existence of a chemotactic stimulus that originated in that area or close to it, which could initiate and sustain the recruitment of inflammatory cells. Considering that the leakage of bacteria and/or fluids at the I-A interface has been previously demonstrated [2,3,7,11,16-18],



Figure 1: Solution containing *Escherichia coli* that was inoculated into the internal part of the implant.



Figure 2: Application of the torque recommended by the manufacturer to the screw of the prosthetic abutment.



Figure 3: Microbrush dampened in saline solution spread on the implant platform in order to identify potential accidental contamination.

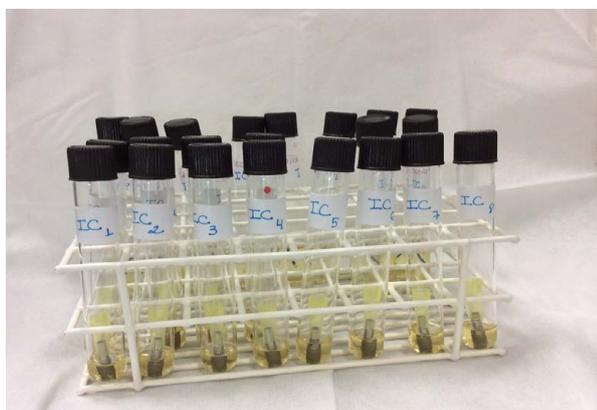


Figure 4: Implants immersed in the culture medium (BHI).

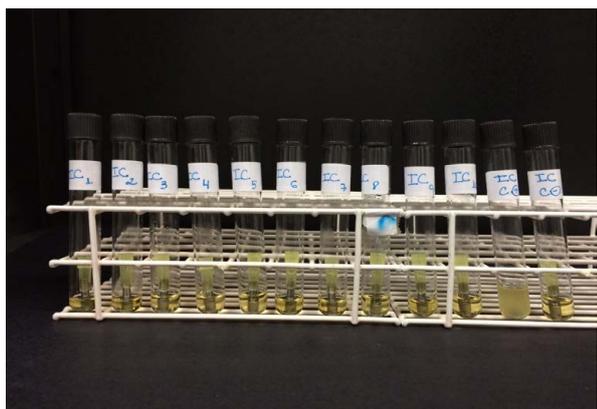


Figure 5: Group 1 samples after 14 days of incubation, showing turbidity only in the positive control samples.

it seems plausible that microorganisms in the oral cavity might be lodged at the I-A interface and be responsible for the stimulus that encourages the growth and activation of osteoclasts, resulting in bone loss around the implant. Bone loss is especially serious in the case of short implants (7 mm) such as those tested in this study. However, no sample in Group 1 showed bacterial leakage, which favors its clinical use. Group 2 showed bacterial leakage at the prosthetic I-A interface

in only one sample. These results are in agreement with those obtained in a previous study involving external hexagon implants [4], which also failed to find bacterial leakage in 4 out of the 5 systems tested. External hex still is the most used implant platform design and some studies consider it more prone to bacterial leakage through I-A interface [6]. In the present study none of the external hex samples (Group 1) showed bacterial leakage, indicating that it can be safely used when we choose a good implant system.

It was proven that the torque applied to the prosthetic screw might influence bacterial leakage at the prosthetic I-A interface [18-21]. In this study, the torque recommended by manufacturer was applied to the screws in the prosthetic abutments, thus avoiding potential variables that might interfere with the results.

The size of the misfit on the I-A interface might also influence bacterial leakage; however, *in vitro* studies have not shown a direct correlation between the adjustment of the I-A interface and bacterial leakage [3,4].

The microbrush used for implant sample B6 presented a positive result for contamination but the implant showed no contamination during the whole period of observation. We believe that this fact may be due to a very small accidental contamination outside of the implant at the time of inoculation with *E. coli* suspension, which has been removed by the microbrush saline, washing the surface. Thus, there was no bacterial growth on the implant

Several methods have been used to assess the leakage in I-A interfaces, including inoculation of the internal part of the implant with a suspension containing bacteria [3,4,22], immersion in saliva [23], immersion in culture medium containing bacteria [6], and inoculation of the internal part of the implant with a colony of bacteria [24]. In this study, the method chosen was the inoculation of 0.3 μ L of a suspension containing *E. coli*, since this is the method most frequently used in other studies with similar goals, because it is easy to handle in laboratories and has a short proliferation time (about 20 minutes). It can be found in the buccal medium of healthy individuals [3].

Conclusion

The implants in Group 1 (external hexagon) did not show bacterial leakage at the prosthetic I-A interface in any sample, while the implants in Group 2 showed leakage in 1 out of 10 samples tested, demonstrating the benefit of appropriate sealing of the prosthetic I-A interface.

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