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Original Article

Bacterial contamination along implant-abutment interface in external and internal-hex dental implants

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Abstract: The aim of this research was to evaluate bacterial contamination along the implant-abutment interface in relation to the size of the interface. 80 brand name implants were used, 40 internal-hex and 40 external-hex. The implants were handled in a sterile atmosphere inside a box, where they were inoculated with 0.3 µl of the *Streptococcus sanguis* ATCC10556 bacterium in the interior and the abutment was immediately installed with a torque of 30 Ncm for the external-hex and 20 Ncm for the internal-hex; the system was included in an Eppendorf control for 30 seconds and then placed in an Eppendorf control for 30 days. The implants were removed and assessed under a scanning electron microscope while the Eppendorf controls were bred in blood agar to analyze the colonies formed. The data were analyzed using the Chi-squared, Kruskal-Wallis and Mann-Whitney tests, considering a value of $p < 0.05$ to obtain statistical significance. Five implants were excluded due to probable external contamination. Microspaces of up to 86.8 µm were observed in the external-hex implants and up to 53.9 µm in the internal-hex implants with no significant differences between the different systems being observed ($p > 0.05$). The contamination observed was produced mainly in the external-hex implants and statistically significant differences were observed between the different hex systems from the same company. No significant differences were observed between interface size and bacterial contamination. Within our limitations, there was no relation between the size of the implant-abutment interface and bacterial contamination with *Streptococcus sanguis* ATCC10556.

Keywords: Implant interface, bacterial contamination, dental implant

Introduction

Peri-implantitis is a complication associated with implant loss [1]; it has already been shown that untreated periodontal alterations, systemic or immunological alterations, a smoking habit and mechanical factors all contribute to implant bone loss and ultimately to the early loss of osseointegration [2]. This condition creates tremendous complications later on because new implant installation options involve prior reconstructive procedures and modification of predisposing factors [3].

In two-phase implant systems, different levels of adaptation between the abutment and implant have been observed, which may cast doubt

on their marginal stability [4]. Quirynen and Van Steenberghe [5] indicated that the likely contamination of the inside of the implant occurs when microorganisms cross over from the periphery towards its interior, while Vidigal Jr. [6] observed a variation of 20 to 150 µm in the implant-abutment interface that could be associated with bacterial contamination. Rodríguez and Baena [7] reported that the type of implant surface might also influence the adhesion of microorganisms.

Gross [8] found microspaces of varying sizes in five different brand name implants and in spite of this, King [9], using radiographic analysis, reported that the interface size might not have any significant influence on the migration of the

Table 1. Number and percentage of contaminated implants according to the brand name and hex type used

| Brand name | Hex | | <i>p</i> -value |
|-----------------|-----------|-----------|-----------------|
| | External | Internal | |
| Conexão® | 2 (22.2%) | 5 (62.5%) | 0.153 |
| INP® | 9 (90.0%) | 6 (66.7%) | 0.153 |
| Neodent® | 2 (22.2%) | 0 | 0.211 |
| Serson® | 0 | 1 (10.0%) | 0.104 |
| <i>p</i> -value | 0.001* | 0.002* | |

*Positive statistical differences.

bone crest towards the apical crest in two-phase implants.

Considering these factors, the discrepancy between the abutment and the implant platform is a risk factor for the short-term stability of microbiological parameters and long-term clinical parameters [10, 11] such that it is necessary to recognize the presence of spaces between abutments and implants.

Materials and methods

80 implants were used from the following brand names: Conexão Sistema de Prótese® (Sao Paulo, Brazil), INP® (Sao Paulo, Brazil), Neodent® (Curitiba, Brazil) and Serson® (Sao Paulo, Brazil). All were made of commercially pure titanium; 40 had external-hex systems and 40 internal-hex systems, together with UCLA abutments for each implant. The sample was divided into eight groups according to the brand and hex type, with 10 implants in each group.

Microbiological analysis

The implants and the abutment system were fully sterilized according to the manufacturer's instructions. The stages of the experimental model were performed at 5 different points on different days due to the sensitivity required by the operator; 16 implants were included in each phase, using 2 implants from each group at every point of analysis until completing the 80 implants, all of them examined under the same conditions.

The bacteria selected for the analysis were *Streptococcus sanguis* ATCC (24 10556 SS-ATCC), which was activated 24 h before each experiment using 100 µl of the previously defrosted strain and breeding it in BHI culture (Brain Heart Infusion, Bioline, Milan, Italy), and

incubating it at 37°C for 24 h in a bacteriological incubator (Biomatic, Porto Alegre, RS, Brazil). The purity of the growth obtained in the BHI culture medium was verified by Gram coloration in sheep blood agar for 48 h at 37°C, confirming the existence of colonies with the same morphology compatible with the microorganism used.

Next, the experiment was performed inside an aseptic box (using sterile material), for which a clamp with a 10 mm mandrel was used (Makita®, Makita do Brasil Ferramentas Elétricas Ltda.) to keep the implant vertical. Then, using a precision pipette, 0.3 µl of *Streptococcus sanguis* ATCC10556 bacteria were inoculated (BHI solution contaminated with SS-ATCC), which had been previously activated in the manner described. The solution was applied to the inside of the implant without contaminating its external surface.

The abutments were then torqued with 20 Ncm for the internal-hex implants and 30 Ncm for the external-hex implants according to the manufacturer's instructions. In a completely sterile environment, to confirm possible external contamination, the system was introduced in a (control) Eppendorf containing 1.5 ml of BHI culture medium for 30 seconds; later the implant was removed and placed in another Eppendorf for 30 days. In the first Eppendorf reading, 24 h from the start of the experiment, coloration of the medium was observed to confirm the absence of contamination and if there was contamination, the implant was removed from the study for probable external contamination or failure of the sterilization system.

Once the experiment was finished, the contaminated material in the Eppendorf was removed to prepare the slides with Gram stain and also to be bred in a Petri dish in blood agar to perform the catalase test, thus confirming the morphology and characteristics of the contaminating microorganism.

The implants contaminated in the first 24 h were excluded from the study as were those contaminated by bacteria different from the one inoculated at the start of the experiment. All the assessments were performed by the same observer to homogenize criteria using clean, uncontaminated eppendorfs as a means of comparison in the initial stage, together with the routine technique of eppendorf.

Table 2. Average, minimum and maximum values (µm) of the microspaces obtained in the interface between the abutment and implant platform

| Brand name | Connection interface | | | | | |
|------------|----------------------|--------------|--------------|--------------|--------------|--------------|
| | External-hex | | | Internal-hex | | |
| | Average | Minimum (µm) | Maximum (µm) | Average | Minimum (µm) | Maximum (µm) |
| Conexão® | 9.65aA | 5.50 | 86.8 | 9.02aA | 4.50 | 18.45 |
| INP® | 9.32aA | 2.25 | 22.85 | 13.30aA | 6.15 | 18.30 |
| Neodent® | 6.70aA | 2.95 | 10.00 | 4.20bA | 1.30 | 11.40 |
| Serson® | 7.7aA | 4.00 | 11.60 | 9.1abA | 0.00 | 53.90 |

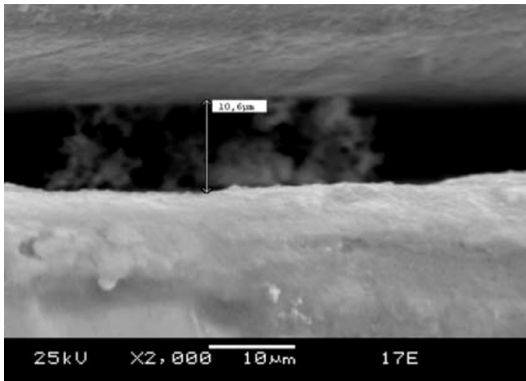


Figure 1. External-hex implant interface with the greatest space observed being 10.6 µm.

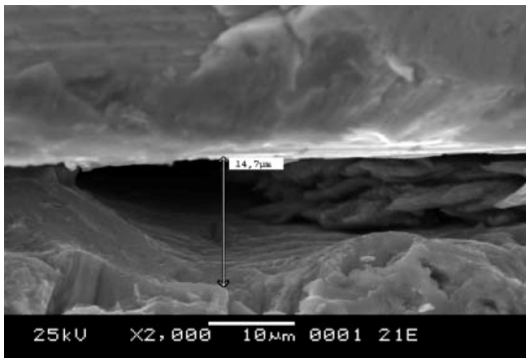


Figure 2. External-hex implant interface with the greatest space observed being 14.7 µm; shape alterations were also observed in the interface.

Analysis with scanning electron microscope

The implants used were removed, dried for two hours and measurements were taken between the abutment and the implant in a Joel JSM 5600 LV scanning electron microscope (SEM), using magnifications of 25x to 2500x. General and by-sector observations were made measuring the smallest and largest spaces. The

data were analyzed in the software Stata v. 9.0 using Chi-squared, KruskalWallis and Mann-Whitney tests, considering a value of $p < 0.05$ to obtain statistical significance.

Results

Five implants were excluded from the study due to external contamination of the culture medium on the first day; this contamination was likely by staphylococci, bacilli and diplococci. For the analysis, 38 external-hex and 37 internal-hex samples were viable.

Contamination observed in different systems presented variability and only the Serson® HE and Neodent® HI implants were free from bacterial contamination. A statistically significant difference was observed among the contamination in the HE and HI implants of the same brand, but no significant differences were observed between the different companies in the microbial contamination analysis (**Table 1**). The systems with the highest contamination were the INP® HE system with 9 contaminated units and the INP® HI system with 6 contaminated units.

When the images were analyzed with SEM, differences were observed between the different systems and companies (**Table 2**); external-hex implants also presented the largest interface with the greatest irregularity (**Figures 1-3**). The interface with internal-hex implants were more regular and smaller (**Figures 4 and 5**); nevertheless, the Kruskal-Wallis and Mann-Whitney tests showed significant differences in the spaces observed in the different systems ($p < 0.05$). Similarly, the Spearman test did not reveal any statistically significant association between the microspaces observed and the contamination of the implants (**Table 3**).

Discussion

In 1989, Nakazato [12] conducted an experiment where bacterial colonization was confirmed after 4 h of exposure, while Koka [13] confirmed that subgingival bacterial colonization in implants presented at 14 days after the insertion of the abutment so that the time to

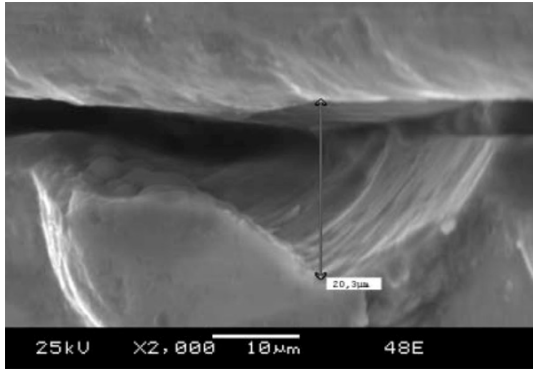


Figure 3. External-hex implant interface with the greatest space observed being 20.9 μm .

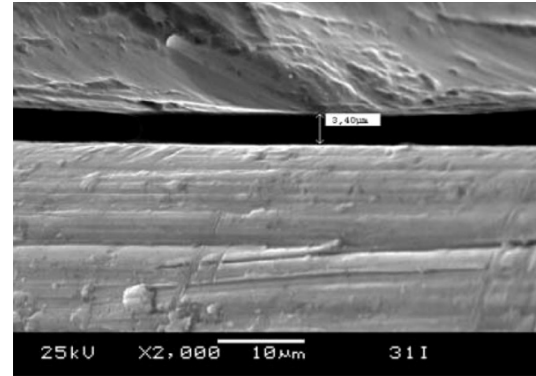


Figure 5. Internal-hex implant interface with the greatest microspace observed being 3.4 μm .

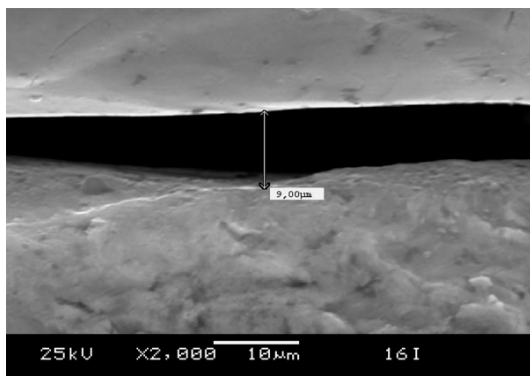


Figure 4. Internal-hex implant interface with the greatest microspace observed being 9.0 μm .

contamination in this study is greater than that found in other investigations and covers the aims of this study.

The *Streptococcus sanguis* ATCC 10556 species was selected due to its high affinity and capacity for adhering to titanium; moreover, as one of the dental biofilm colonizers, it is 1 to 0.8 μm on average, which might help its intervention in the peri-implantitis flora [14]. Wolinski [15] evaluated the adhesion of *Streptococcus sanguis* G9-B and *Actinomyces viscosus* T14V to dental enamel surface and commercially pure titanium implants and in both *Streptococcus sanguis* had the greatest adhesion. Testing different microbial species, Edgerton [16] also indicated that *Streptococcus sanguis* and *Streptococcus oralis* had the strongest bond compared to the other species.

Microspaces between the abutment and the implant platform are inevitable and typical of

the manufacture of the implants; although the typical oral bacteria measure 0.5 to 2 μm [14], spaces up to 51 μm between abutment and implant are considered acceptable. Previous studies by Vidigal Jr. [6] compared the adaptation of different systems and demonstrated variations of 50 μm for the SR-Press system, 150 μm for the Tissue Functional (TF) system and 20 μm for the Branemark system. Dellow [17] found values from 0 to 7.15 μm and Callan [18] values of 30 to 135 μm . Our results showed microspaces of up to 86.8 μm in the external-hex implants and up to 53.9 μm in the internal-hex implants without observing significant differences among the different systems ($p>0.05$).

Do Nascimento [4], in a study of characteristics similar to ours, demonstrated contamination in internal-hex implants bound to premachined and cast connectors with no differences between the two. Silva-Neto [19] showed that bacterial leakage was independent of abutment insertion torque, although when the torque was 32 Ncm, no bacterial leakage was observed in the implants. Our study use an insertion torque of 30 Ncm and 20 Ncm, revealing leakage in the different systems evaluated with no statistical differences between them. Additionally, Ricomini [20] reported that the installation of abutments subjected to fatigue and load presented a higher torque value on removal and were not related to the bacterial contamination to which they were subjected. Likewise, Jansen [21] compared the size of the spaces and the proportion of contamination and found no statistically significant relation between the size of the microspaces and the proportion of bacterial

Table 3. Correlation between the average adaptation values (μm) and the contamination observed in the experimental model

| Brand name | Hex | | | | | |
|------------|--------------|----------------------|---------|--------------|----------------------|---------|
| | External-hex | | | Internal-hex | | |
| | n | Spearman coefficient | p-value | n | Spearman coefficient | p-value |
| Conexão® | 9 | 0.091 | 0.809 | 8 | 0.268 | 0.498 |
| INP® | 10 | -0.045 | 0.892 | 9 | 0.017 | 0.498 |
| Neodent® | 9 | 0.068 | 0.844 | 10 | - | - |
| Serson® | 10 | - | - | 10 | 0.174 | 0.607 |

leakage; for example, implants like Astra Tech® and Bonefit® exhibited good indicators of adaptation but high contamination levels.

Jaworski [22] indicated low contamination of Morse taper connection implants compared to external-hex implants, and similar conclusions were drawn by Koutouzis [23], who indicated that Morse taper connection implants present less contamination in the interface than internal connection implants, suggesting that the design of the implant also plays a role in implant-abutment interface contamination, which was not observed in our results that compared external and internal-hex connections.

Despite finding considerable variability in the contamination of different implants, in this study no statistical relation was found between the microspaces of each implant (hex types and brand names) and the contamination observed. Therefore, we can agree that the loss of cervical bone and peri-implantitis can also be associated with other factors such as diet, occlusion, angulation of the implant, periodontal disease, smoking habit, among others [24-26].

Finally, we can conclude that the implants evaluated do not present statistically significant differences in terms of bacterial contamination with *Streptococcus sanguis* ATCC10556 and the size of the microspaces present in the implant-abutment interface.

Disclosure of conflict of interest

The authors declare that they have no competing financial interests.

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