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IMPLANT-ABUTMENT LEAKING OF REPLACE CONICAL CONNECTION NOBEL BIOCARE® IMPLANT SYSTEM. AN *IN VITRO* STUDY OF THE MICROBIOLOGICAL PENETRATION FROM EXTERNAL ENVIRONMENT TO IMPLANT-ABUTMENT SPACE

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SUMMARY

Purpose. The aim of our study is to value the microbial contamination in the implant-abutment connections (IAC) of a Nobel Replace Conical Connection implant system [Nobel Biocare®, Vimercate (MB), Italy].

Materials and methods. To identify the capability of the implant to protect the internal space from the external environment, the passage of genetically modified bacteria across IAC was evaluated. Four Nobel Replace Conical Connection implants (Nobel Biocare®, Vimercate (MB), Italy) were immerged in a bacterial culture for twenty-four hours and then bacteria amount was measured inside and outside IAC with Real-time PCR. Bacterial quantification was performed by Real-Time Polymerase Chain Reaction using the absolute quantification with the standard curve method.

Results. In all tested implants, bacteria were found in the inner side, with a median percentage of 10.9%.
The analysis revealed that in both cases (internally and externally), bacteria grew for the first 48 hours but subsequently they started to dye, probably as a consequence of nutrient consumption. Moreover, the difference between outer and inner bacteria concentration was statistically significant at each time point.

Conclusions. Implant's internal contamination shows that IAC is not sealing.
The reported results are similar to those of previous studies carried out on different implant systems. Until now, no IAC has been proven to seal the gap between implant and abutment.

Key words: bone resorption, implant-abutment connection, microbiological leakage, and perimplantitis.

Introduction

Oral rehabilitation with implants has become one of the most successful dentistry techniques over the last 20 years (1, 2). The success rate of implant dentistry is above 80%. However, peri-implantitis is the most important complication of implant dentistry.

In addition, the success of implant rehabilitation is related to mechanical properties, such as the

implant-abutment connection (IAC) and the correct loading (1). The occlusal overloading after prosthetics can result in stress increase in the implant and in implant-abutment connection, as well as in the surrounding bone (3). A wrong fit of IAC could cause screw fracture and loss, prosthetics damage and unfavorable patient compliance.
IAC is also influenced by biological factors. The success of dental implants depends on osseointegration phenomena and bone level maintenance around implants (3-5). In addiction the presence of oral diseases such as periodontal disease, atrophy of the oral mucosa, lesions of gastroesophageal reflux or oral lichen planus may increase the risk of peri-implantitis (6-10).
The presence of a micro-gap at the IAC allows microorganisms to penetrate and colonize the inner part of the implant leading to biofilm accumulation and consequently to peri-implantitis development (11).
The IAC has an important role in the onset of peri-implantitis. The presence of a gap in IAC is associated with a significantly higher inflammatory cell infiltration and bone loss (12).
In fact, some minutes after implant placement, bacterial colonization of implant surfaces and peri-implant tissues, immediately starts (13). The connection between abutment and implant creates a gap resulting in bacterial leakage and in an area of inflamed soft tissue around the IAC (14). Prevention of microbial leakage at the level of IAC is the main aim for the construction of two-piece implant systems to avoid inflammation in peri-implant tissues. Microbial leakage is an important factor for chronic inflammatory infiltration and marginal bone resorption. New designs of IAC aim to improve precise tight mechanical connection and thus minimize the bacterial leakage. This process is technically very difficult as bacteria are around 1- 10μm in length. The goal of our study is to value the microbial leakage in the IAC of the Nobel Replace Conical Connection implant system [Nobel Biocare®, Vimercate (MB), Italy] (Figure 1).

Nobel Replace Conical Connection implant system

Nobel Replace Conical Connection implant system [Nobel Biocare®, Vimercate (MB), Italy] combines the original tapered implant body with a sealed conical connection, providing an aesthetic solution for all indications. The implant body reproduces the shape of the natural tooth root, ensuring high initial stability for all load protocols, including the immediate loading.
The implant-abutment interface is critical to a functional outcome and long lasting aesthetic. The internal conical connection has a sealed connection and a high mechanical resistance. This characteristic ensures the necessary stability for a predictable prosthetic result.

Materials and methods

Implant preparation

In order to size up the ability of the implant to isolate the heart of the device from the external environment, we evaluated the passage of modified bacteria across the joint of the implant. The peculiarity of these bacteria is that they contain synthetic DNA target sequences in their plasmid. In detail, the broth contains two bacterial species (*P. gingivalis* and *T. forsythia*) and two plasmids for antibiotic selection (Kanamycin and Ampicillin).
Bacteria were cultured in lysogeny broth (LB) containing both Kanamycin and Ampicillin (at a final concentration of 50ug/ml) at 37°C for 12-18h in a shacking incubator. Four Nobel Biocare® implants were used in this study (Figure 1a). Few microliters of LB with antibiotics were put inside the implants (Figure 1b). The implants and the abutment are screwed with a force of 35 newton (Figure 1c, d).
Few microliters of this culture were used to "contaminate" fresh LB with antibiotics contained in a

Figure 1
a) Four implants Nobel Replace Conical Connection implant system [Nobel Biocare®, Vimercate (MB), Italy]. b) Few microliters of LB with antibiotics were put inside the implants. c-d) The implant and the abutment are screwed with a force of 35 newton. e) Tubes let at 37°C for 48h in a heater, in order to allow bacterial growth and their hypothetical passage within the implant. Inside the implant, instead, were just put LB and antibiotics without bacteria. f) Implants opened and samples collected by dipping a paper probe in both the sites containing LB (external and internal to the implant) for each implant, and in the negative control too forty-eight hours later.

microcentrifuge tube together with the implant. Tubes were then let at 37°C for 48h in a heater, in order to allow bacterial growth and their hypothetical passage within the implant (Figure 1e). Inside the implant, instead, we just put LB and antibiotics without bacteria.

To be sure that there were no contaminations, a negative control containing only LB and antibiotics was prepared.

Forty-eight hours later, implants were opened and samples were collected by dipping a paper probe in both the sites containing LB (external and internal to the implant) for each implant, and in the negative control too (Figure 1f).

DNA extraction

Once collected, paper probe were put on a new microcentrifuge tube and processed for bacterial DNA extraction, by using the GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, St., St. Louis, MO, USA), following the manufacturing procedures. Briefly, samples were incubated with lysozyme and, subsequently with proteinase K to isolate DNA. Once extracted, DNA was purified by spin-column method.

Real-time polymerase chain reaction

Bacterial quantification was performed by Real-Time Polymerase Chain Reaction using the absolute quantification with the standard curve method.
Primers and probes oligonucleotides for *P. gingivalis* and *T. forsythia* were designed basing on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1).
For the quantitative analysis, plasmid (Eurofin MWG Operon, Ebersberg Germany) containing the specific DNA target sequence was employed as standard.
All reactions were performed in duplex, in 20ul final volumes, with 2X TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA, USA) and 50nM concentration of each primers and 200nM of the probes. Amplifications were carried out by using the ABI PRISM 7500 (Applied Bio systems, Foster City, CA, USA).

Statistical analysis

To evaluate if the difference in viability among outside and inside the implant was statistically significant, we applied Student's t-test on average bacteria quantification at each time point.

Results

Bacteria quantification is reported in Table 1. In all the tested implants, bacteria were found in the inner side, with a median percentage of 10.9%.
The analysis revealed that in both cases (internally and externally), bacteria grew for the first 48 hours but subsequently they started to dye, probably as a consequence of nutrient consumption. Moreover, the difference between outer and inner bacteria concentration was statistically significant at each time point.

Table 1 - Bacterial quantification and calculation of their entry's percentage.

	Outside	**Inside**	
Implant	*P. Gingivalis + T. forsythia* Absolute Quantification	*P. Gingivalis + T. forsythia* Absolute Quantification	% bacterial entry
N1	484597	3519	0,7
N2	1198863	10591	0,9
N3	60562	19437	32,1
Negative Control	0	0	0

Discussion

Prevention of microbial leakage at the level of IAC is the main aim for the construction of two-piece implant systems to avoid inflammation in peri-implant tissues.
The design of IAC can limit the microbial penetration into the internal part of a dental implant (14). Some microbiological studies confirmed the passage of bacteria around IAC at level of peri-implant tissues (15). An *in vitro* study (15) showed microbial penetration of the IAC micro-gap of fixtures with an external hex design. In addition, it is also known that such diseases like oral lichen planus, oral dysplastic lesions, and burning mouth syndrome may favour the onset of peri-implantitis (16-19).
Other Authors (20) studied microbial leakage in different implant-abutment connections, showing microbial contamination in implant with an internal connection. Another study (14) evaluated bacterial penetration along the IAC micro-gap and established bacterial colonization in an *in vitro* experiment using loading forces.
Other studies (20-22) have investigated bacterial leakage in order to find an efficient bacterial seal system. The two-piece implant system inevitably leads to the presence of cavities between implant and abutment favoring an inflammatory process in peri-implant tissues. Microbial colonization of the IAC may have consequences as bone resorption. Tesmer, in an *in vitro* study has demonstrated the passage of fluid into and out of IAC (20). Similar results were obtained in another study showing bacterial penetration on the internal surfaces of system implants (22).
Our results are similar to those reported in the English literature. Aloise et al. found that the frequency of bacterial leakage along the implant-abutment interface was 20% of the assemblies of Bicon© and Ankylos systems (21). Implant internal contamination evidently shows that the presence of micro-cavities in IAC may represent a bacterial passage from the external medium (21). In a recent *in vitro* study, do Nascimento et al. (14) demonstrated a similar bacterial infiltration through the interface of different implants system. Passage of microorganisms through the IAC has also been shown in other *in vivo* studies (23, 24).
In literature several IAC were examinated, and none demonstrated to prevent microbiological leakage in the inner part of IAC (12).
Some histological studies demonstrate that passage of bacteria at the level of the IAC is the cause of inflammatory reactions of the peri-implant tissues. Broggini et al. (3) demonstrated an increase in inflammatory cells at the level of IAC and soft tissues around two-piece implants. This had been responsible for the onset and failure treatment of peri-implantitis. The inflammatory content may increase as a consequence of the adhesion and proliferation of bacteria on the biofilm around IAC during soft tissue manipulation for prosthetic component installation. The presence of a cavity near to bone may influence in the development of peri-implant inflammation and bone resorption. In fact, bacterial leakage could cause an inflammatory process in the peri-implant tissues at the alveolar bone crest level and bacterial infection can interfere with osseointegration healing. Broggini et al. (3) demonstrated an increase in inflammatory cells in the peri-implant soft tissues at the level of IAC.
An intense inflammatory cell infiltrate may be the cause of a significant bone resorption at IAC level. This assumption is confirmed by the fact that one-piece implants showed a minimal inflammation and bone loss around peri-implant tissues. Hermann et al. (25) reported similar results. They demonstrated that the presence of a micro-gap significantly influence hard and soft tissues around an implant. In addition, Hermann et al. (23), in dog experimental studies, observed that there is less bone loss if the IAC is placed coronally away from the alveolar crest. On the contrary, if the micro-gap was moved in an apical direction, a greater amount of bone loss was observed (23, 24). Piattelli found similar results in a retrospective histologic evaluation in monkeys on the role of the micro gap between implant and abutment (26). Few literature data are

available about the differences in the microbial penetration in IAC with different connection designs. The design of the implant-abutment junction may have an impact on the amount of bacterial penetration in the internal part of dental implants. Implant-abutment connection leaking is also influenced by prosthetic (27-30) and endodontic clinical outcome (31-34).

Conclusions

The reported results are similar to previous works. Nobel Replace Conical Connection implant system [Nobel Biocare®, Vimercate (MB), Italy] showed bacterial leakage along IAC lower than others implants systems (10,9 *versus* 20% of Bicon© and Ankylos® systems). In fact, even if the main factor for survival rate of implants is the quality of bone of receiving sites, the bacteria of peri-implantitis may be the main cause of failure of implants. In spite of the limits of our study, none IAC has been demonstrated to perfectly close the gap between implant and abutment.

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