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RESEARCH & DEVELOPMENT

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SUMMARY

Purpose: The aim of the present study is to evaluate the effectiveness of Ditron's implant and abutment connection *(IAC)* in sealing the gap between these two parts.

Materials and methods: To identify the efficacy of a new IAC, the passage of genetically modified Escherichia coli across IAC was evaluated. A total of five Ditron implants were used. All implants were immerged in a bacterial culture for forty-eight hours and then the amount of bacteria 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 the tested implants, bacteria were found in the inner side with a median percentage of 1.35%. The analysis revealed that, in untreated implants, bacteria grew *(internally and externally)* for the first forty-eight hours, but subsequently, they started to die. Moreover, the difference between outer and inner bacteria concentration was statistically significant at each time point.

Conclusions: Ditron Implant IAC *(MPI, Ditron Dental, Israel)* is effective in reducing bacterial leakage.

INTRODUCTION

Osseointegrated dental implants showed elevated success rates in long-term treatment in the last ten years[1]. These rates referred to primary stability, and in turn, related to the quantity and quality of the receiving bone [1]. However, despite the high success rates in the long-term, the risk of peri-implantitis and implant failure is the main complication of implantology [2]. There are many hypothesized causes of peri-implantitis, but bacterial

colonization at the implant-abutment connection *(IAC)* is the most accredited one[3]. The presence of a micro-gap at the IAC allows micro-organisms to penetrate and colonize the inner part of the implant leading to biofilm accumulation and consequently to peri-implantitis development[4-7]. The bacterial colonization at IAC level has an important role in the onset of peri-implantitis[5]. In addition, the presence of oral diseases such as peri-odontal disease, atrophy of the oral mucosa, lesions of gastroesophageal reflux or oral lichen planus may increase the risk peri-implantitis[6-12].

The presence of a gap in IAC is associated with a significantly higher inflammatory cell infiltration and bone loss[13]. In fact, some minutes after implant placement, bacterial colonization of implant surfaces and peri-implant tissues immediately starts. The connection between abutment and implant creates a gap resulting in bacterial leakage and in an area of inflamed soft tissue around the IAC. 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. This assumption is confirmed by the fact that the percentage of peri-implantitis is minimized in one piece implants, where there is no IAC and no bacteria leakage[13].

Several in vitro studies have demonstrated that, even if different internal connection designs were proposed, not one prevented the passage of bacteria along IAC in static or dynamic loading conditions[14]. Micro-leakage has been confirmed bidirectional, from the inner parts of the implants to the external environment and vice versa[15]. Some reports have demonstrated that the use of sealing materials, decontamination of the inner-implant cavity, use of shape memory alloy and different connection geometries, have been unsuccessful in prevent bacterial leakage. It is also known that such diseases like oral mucositis, oral dysplastic lesions, and burning mouth syndrome may favour the onset of peri-implantitis[16-18].

Some studies tried to quantify microbiological penetration between micro-gaps at IAC level, all concluding that no one IAC has been demonstrated to perfectly close the gap between implant and abutment, favoring the one set and maintenance of peri-implantitis[19]. Limited success has been achieved in eliminating the implant–abutment gap or simply avoiding its effects. Some different solutions, such as inclusion of polymeric washers between the parts of different implant systems, only decreased, but did not eliminate, bacterial contamination[20].

MATERIALS AND METHODS

MOLECULAR PRECISION IMPLANT (MPI) CHARACTERISTICS

MPI *(Molecular Precision Implants, Ditron Dental, Israel)*: Due to its sophisticated control system of the surfaces, the MPI provides the implantologist a safe and reliable implant, with a macro-morphology designed to ensure a close contact with the surrounding bone.

The characteristics of this new implant are: MolecuLock™ *(seal between implant and abutment; biomechanical design and 1$_\mu$ level production to reduce micro gaps and micro movement risks)*, surface treatment *(Al2O3 surface blasting and double-acid etching; high purity cleaning procedures)*, implant body *(high initial stability even in compromised bone situations; expanding tapered implant body with double-thread self-tapping design condensing bone gradually to enhance primary stability; insertion rate of the Molecular Precision Implants of 2.2$_{mm}$ per revolution)*, restorative platform *(a beveled collar shifting the implant-abutment junction inward in order to achieve platform-switching configuration; platform switching - generating a perfect environment for the soft tissue growth and helps prevent bone resorption)*, assisted osteointegration *(Unique Spherical Helix Chamber forming a localized infrastructure that serves as a scaffold for promoting wound healing and bone formation from existing osteoblasts)*, Apex design *(Apex with self-*

tapping drilling blades enables smaller osteotomy; the self-tapping function supporting a precise adaptation of the implant thread to the bone, thus providing optimal primary stability; improved ease of insertion and allowing mild direction refinement during the initial stages of insertion).

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 E. coli 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 plasmid contains two sequences specific for two bacterial species *(P. gingivalis and T. forsythia)* and two genes 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 12-18hr in a shaking incubator. Five molecular precision implants *(MPI, Ditron Dental, Israel)* were used in this study. A few microliters of LB with antibiotics were put inside the implants *(Figure 1A)*. The implants and the abutment were screwed with a force of 35 Newton *(Figure 1B)*. A few microliters of this culture were used to "contaminate" fresh LB with antibiotics contained in a microcentrifuge tube together with the implant. Tubes were then left at 37°C for forty-eight hours in a heater, in order to allow bacterial growth and their hypothetical passage within the implant *(Figure 1C)*. 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 sites containing LB (external and internal to the implant) for each implant, and in the negative control too *(Figure 1D)*.

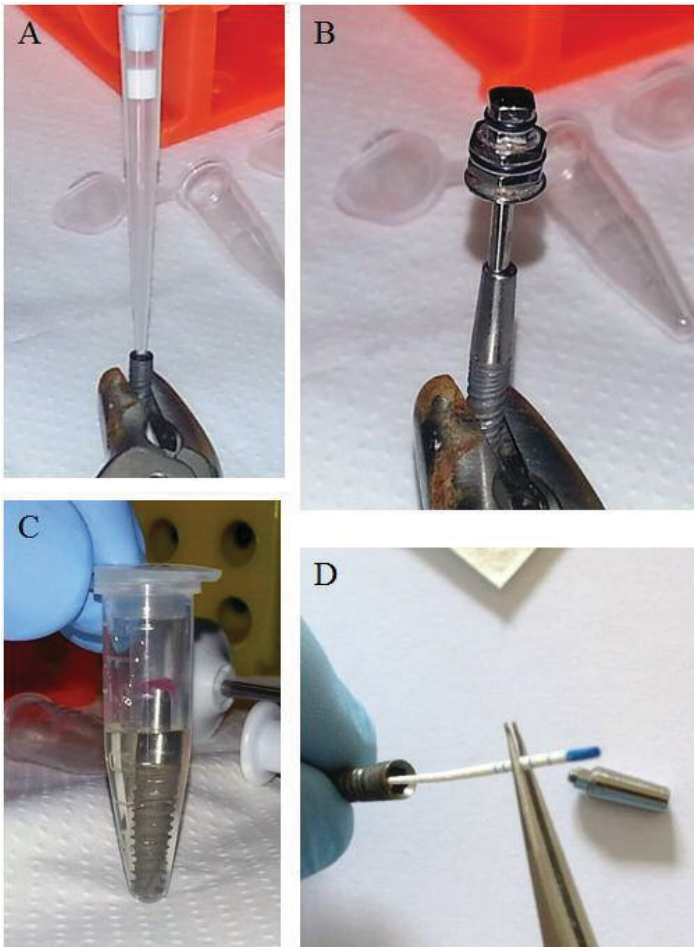

Fig. 1:
(A) Few microliters of LB with antibiotics were put inside the implants;
(B) The implant and the abutment were screwed with a force of 35 Newton;
(C) Tubes left at 37 °C for 48h in a heater, in order to allow bacterial growth and their hypothetical passage within the implant. Inside the implantonly LB and antibiotics were put without bacteria;
(D) 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.

DNA EXTRACTION

Once collected, a paper probe was put on a new microcentrifuge tube and processed for bacterial DNA extraction by using the GenEluteTM 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 based on 16S rRNA gene sequences of the Human Oral Micro-biome 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 Biosystems, 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 1.35%. The analysis revealed that in both cases *(internally and externally)*, bacteria grew for the first forty-eight hours but, subsequently, they started to die, probably as a consequence of nutrient consumption. Moreover, the difference between outer and inner bacteria concentration was statistically significant at each time point.

	OUTSIDE	**INSIDE**	
IMPLANT	P. Gingivalis + T. forsythia Absolute Quantification	P. Gingivalis + T. forsythia Absolute Quantification	Passage of bacteria from outside to inside the implant (%)
DITRON 1	922154	35420	3,8
DITRON 2	372115	0	0,0
DITRON 3	790818	9743	1,2
DITRON 4	1000226	4214	0,4
Negative Control	0	0	0

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

DISCUSSION

It is clear that peri-implantitis occurs due to the presence of pathogenic micro-organisms colonizng the surrounding implant area, and the suppression or eradication of these microbes results in prevention of peri-implantitis[1]. The main cause of peri-implantitis consists in the passage of pathogenic bacteria in the abutment-implant gap. The inner spaces were easily colonized, and bacteria may leak out from these spaces through the IAC into the peri-implant area. The formation of a biofilm around the implant plays a fundamental role in the onset of peri-implantitis[21]. In any case, the peri-implantitis is associated with gram-negative bacteria similar to those that cause periodontal disease[3]. The peri-implantitis, such as periodontal disease, is the result of the bacterial influx and the subsequent host response. Some studies have shown that bacterial species of periodontal disease are very similar to those that cause peri-implantitis[19]. For which it is clear that blocking the passage of bacteria in the peri-implant space is essential to prevent peri-implantitis. The use of a new implant-abutment connection can represent a valid solution to prevent the development of peri-implantitis. The potential benefits of a new IAC include improved patient compliance and an easier access to implant-abutment space[20-26]. The use of this new IAC may influence prosthodontic[27-30] and endodontic[31, 32] clinical outcomes. In addition, the use of general and local anesthesia may have side effects[33-36] and severe complications[37].

The adoption of a new IAC has been demonstrated to dramatically reduce the bacterial leakage. This new IAC shows reduced bacterial leakage for most perio-pathogens, and at the same time, exhibits negligible impact on the microflora residing in other parts of the body.

Our study evaluated the efficacy of this new IAC in preventing bacterial leakage infiltrated between implant and abutment, considered the primary etiological factors for peri-implantitis.

Microbiological testing was thought appropriate to evaluate the effect of this new IAC on subgingival microbial population. It is well known that both P. gingivalis and T. forsythia occur concomitantly with the clinical signs of bone resorption surrounding implant. They appear closely 'linked' topologically in the developing biofilm, exhibiting an in vitro ability to produce a number of outer membrane-associated proteinases. They are considered the first pathogens involved in the clinical destruction of peri-implant bone and in the local development of peri-implantitis. Our results demonstrated that this new IAC performs very well in preventing bacteria proliferation of the microbial species which comes in contact with it. Molecular precision implant-abutment connection *(MPI, Ditron Dental, Israel)* is efficacy in reducing bacterial leakage. 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, no IAC has been demonstrated to perfectly close the gap between implant and abutment.

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