ORAL & IMPLANTOLOGY

original research article

A NEW SYSTEM OF IMPLANT ABUTMENT CONNECTION: HOW TO IMPROVE A TWO PIECE IMPLANT SYSTEM SEALING

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SUMMARY
Purpose. Implant dentistry has become one of the most successful dentistry techniques for replacing missing teeth. The success rate of implant dentistry is above 80%. However, peri-implantitis is a later complication of implant dentistry that if untreated, can lead to implant loss. One of the hypotized causes of peri-implantis is the bacterial leakage at the level of implant-abutment connection. Bacterial leakage is favored to the presence of a micro gap at the implant-abutment interface, allowing microorganisms to penetrate and colonize the inner part of the implant leading to biofilm accumulation and consequently to peri-implantitis development.
Materials and methods. To identify the capability of the implant to protect the internal space from the external environment, the passage of genetically modified Escherichia coli across implant-abutment interface was evaluated. Implants were immerged in a bacterial culture for twenty-four hours and then bacteria amount was measured inside implant-abutment interface with Real-time PCR.
Results. Bacteria were detected inside all studied implants, with a median percentage of 9%.
Conclusions. The reported results are better to those of previous studies carried out on different implant systems. Until now, none implant-abutment system has been proven to seal the gap between implant and abutment.

Key words: implant-abutment connection, implant dentistry, bacterial leakage, peri-implantitis, bone resorption.

Introduction

Implant dentistry has become one of the most successful dentistry techniques for replacing missing teeth. The success rate of implant dentistry is above 80% (1-16) and implant placement requires an adequate quantity and quality of bone (17-25).
However, peri-implantitis is a later complication of implant dentistry, that if untreated can lead to implant loss.
One of the hypotized causes of peri-implantiis is the bacterial leakage at the level of implant-abutment interface. Bacterial leakage is favored by the presence of a micro gap at the implant-abutment interface level, allowing microorganisms to penetrate and colonize the inner part of the implant leading to biofilm accumulation and consequently to peri-implantitis development (26, 27). Peri-implantitis is associated with a significantly higher inflammatory cell infiltration and bone loss (28). Prevention of microbial leakage at the level of implant-abutment interface is the main aim for the construction of a new two-piece implant systems (TPISs) to avoid inflammation in peri-implant tissues.
The aim of our study is to value the microbial leakage at implant-abutment interface of a new TPIS (Noris Medical Dental Implants System, Israel).

Tuff two-piece implant system

Tuff implant (Noris Medical Dental Implants System, Israel) is a new TPIS, which, with its three thread zones, has been designed according to the anatomy of the bone structure. The lower V-shape thread zone is for self-tapping. The middle zone has a square thread design, used especially for compressing cancellous bone, and helping achieving BIC (Bone-Implant Contact). The micro thread design on the upper zone adds stability and reduces crestal bone loss. Mono implants are specifically indicated for replacing maxillary lateral incisors and mandibular central and lateral incisors. They are cleared for immediate, non-occlusal provisionalization in single-tooth restorations. Multiple-unit restorations should be splinted together and may be used immediately, when clinically appropriate.

The Noris Medical Dental TPIS includes different types and sizes of dental implants made of medical grade Titanium Alloy and undergo a unique surface treatment.

Noris Medical TPIS are used for rehabilitating completely or partially edentulous patients. The rehabilitation on the implants includes a number of options: single crown, a number of connected crowns and partial or full dentures that are connected to Noris Medical TPIS using abutments.

Quantity and quality of bone that are suitable for performing implants are an essential condition. This data is gathered during the planning stage by making appropriate radiographs (panoramic and computer tomography) of the implantation site. Anatomic areas near the implantation site such as: blood vessels, nerves, maxillary sinus and nasal cavity must be identified in order to prevent their damage. The performance of surgical procedures is subject to the patient's systemic condition.

The Noris Medical Dental TPIS employs internal hex connection designed to provide assembly facility while minimizing micro movements of the implant/abutment connection. The implants material composition is: TI 6AL 4V - ELI. The Noris Medical TPIS surface is RBM treated. RBM (Resorbable Blast Media) Surface Technology is a surface treatment processed by blasting the implant with a soluble calcium phosphate material, creating a macro surface roughness, using of biocompatible Calcium Phosphate blasting media. Calcium Phosphates are easily dissolved by gentle solvents like alcohol, leaving well textured surface completely free of contaminants.

Noris Medical Dental TPIS is intended to replace missing tooth/teeth in either jaw for supporting prosthetic devices that may aid in restoring the patient's chewing function. The procedure can be accomplished in a one-stage or two-stage surgical operation. All implants are appropriate for immediate loading when good primary stability is achieved and with appropriate occlusal loading.

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 *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 sequence 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 12-18h in a shacking incubator. Four Tuff implants (Noris Medical®, Israel) were used in this study (Figure 1). Few microliters of LB with antibiotics were put inside the implants. The implants and the abutment are screwed with a torque of 35 Ncm.

Few microliters of this culture were used to "contaminate" fresh LB with antibiotics con-

Figure 1
Tuff Implant and abutment by Noris Medical.

tained in a 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. 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.

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 9%.

Table 1 - Absolute quantification of *P. gingivalis and T. forsythia*, outside and inside the implant. Implant permeability is expressed as percent rate of the internal *vs* external bacteria quantity.

Implant	Bacteria	Bacteria quantity	Implant	Bacteria	Bacteria quantity	Passage of bacteria from outside to inside the implant (%)
1 OUTSIDE	*P. gingivalis*	3581973	1 INSIDE	*P. gingivalis*	697785	19%
	T. forsythia	3304664		*T. forsythia*	708424	21%
2 OUTSIDE	*P. gingivalis*	7195087	2 INSIDE	*P. gingivalis*	396791	6%
	T. forsythia	6789549		*T. forsythia*	400960	6%
3 OUTSIDE	*P. gingivalis*	4579415	3 INSIDE	*P. gingivalis*	1082464	24%
	T. forsythia	4582728		*T. forsythia*	1084939	24%
4 OUTSIDE	*P. gingivalis*	2820289	4 INSIDE	*P. gingivalis*	89335	3%
	T. forsythia	2720166		*T. forsythia*	98433	4%
5 OUTSIDE	*P. gingivalis*	1351250	5 INSIDE	*P. gingivalis*	198973	15%
	T. forsythia	1372971		*T. forsythia*	203651	15%
6 OUTSIDE	*P. gingivalis*	2877517	6 INSIDE	*P. gingivalis*	88918	3%
	T. forsythia	2452891		*T. forsythia*	100066	4%
7 OUTSIDE	*P. gingivalis*	1124582	7 INSIDE	*P. gingivalis*	142005	13%
	T. forsythia	1150407		*T. forsythia*	145277	13%
8 OUTSIDE	*P. gingivalis*	1150527	8 INSIDE	*P. gingivalis*	101557	9%
	T. forsythia	1112707		*T. forsythia*	128467	12%
9 OUTSIDE	*P. gingivalis*	8131886	9 INSIDE	*P. gingivalis*	101248	1%
	T. forsythia	7506339		*T. forsythia*	111292	1%
10 OUTSIDE	*P. gingivalis*	2836594	10 INSIDE	*P. gingivalis*	243945	9%
	T. forsythia	2614350		*T. forsythia*	252896	10%
11 OUTSIDE	*P. gingivalis*	1792653	11 INSIDE	*P. gingivalis*	100353	6%
	T. forsythia	1700109		*T. forsythia*	101758	6%
12 OUTSIDE	*P. gingivalis*	1310796	12 INSIDE	*P. gingivalis*	110644	8%
	T. forsythia	1173590		*T. forsythia*	112948	10%
Negative Control OUTSIDE	*P. gingivalis*	0	Negative Control INSIDE	*P. gingivalis*	0	0
	T. forsythia	0		*T. forsythia*	0	0
	Media Outside			*Media Inside*		
	PorG	3229381		*PorG*	279502	9%
	TanF	3040039		*TanF*	287426	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.

Discussion

Bacterial leakage at implant-abutment connection is the main cause of peri-implantitis. The current TPISs cannot completely prevent microleakage and consequent bacterial colonization of the inner part of the implants. Although efforts have been made to reduce this TPISs limitation, several investigations have shown that bacterial oral leakage along the implant-abutment interface may constitute a potential risk of inflammation of the supporting tissues, compromising the long-term success of the treatment with TPISs. A diversity of data regarding the leakage and consequent bacterial penetration along the gaps and cavities into the TIPSs, as a consequence of poor adaptation of components, has been reported in some *in vitro* studies (26-37).

Other studies demonstrated microbial penetration of the TPISs micro gap of fixtures with an external hex design (29, 30). Some studies (31, 32) have investigated bacterial leakage of TPISs in order to find an efficient bacterial seal system. With the TPISs, the abutment is retained in the fixture with mechanical methods, favoring an inflammatory process in peri-implant tissues. Microbial colonization of the TPISs may have consequences as bone resorption. Some *in vitro* studies has demonstrated the passage of fluid into and out of TPISs. Our results are better to those reported in the English literature (33, 34). Aloise et al. found that the frequency of bacterial leakage was 20% of the TPIS of Bicon© and Ankylos® systems (27). Implant internal contamination evidently shows that the presence of gap in TPISs may represent a bacterial passage from the external medium (35). TPISs do not prevent microbiological leakage in the inner part of implant-abutment interface (36). In any case, the peri-implantitis is associated with gram- negative bacteria similar to those that cause periodontal disease (37). The peri-implantitis, such as periodontal disease, is the result of the bacterial insult and the subsequent host response, in fact some studies have shown that bacterial species of periodontal disease are very similar to those that cause peri-implantitis (38). Blocking the passage of bacteria, in a TPIS is essential to prevent peri-implantitis, in fact the presence of a cavity near to bone may influence in the development of peri-implant inflammation and bone resorption. An intense inflammatory cell infiltrate may be the cause of a significant bone resorption in a TPIS, on the contrary one-piece implants showed a minimal inflammation and bone loss around peri-implant tissues. Some studies demonstrated that the presence of a micro gap significantly influence hard and soft tissues around an implant, so few literature data are available about the differences in the microbial penetration in TPISs 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 of a TPIS system.

Conclusions

The reported results are similar to previous work. Noris Medical Dental Implants System showed bacterial leakage better respect others implant systems (9 *versus* 20% of Bicon© and Ankylos® systems). In spite of the limits of our study, none TPIS has been demonstrated to perfectly close the gap between implant and abutment.

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