

A NEW IMPLANT-ABUTMENT CONNECTION FOR BACTERIAL MICROLEAKAGE PREVENTION: AN *IN VITRO* STUDY

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SUMMARY

Purpose. The aim of our study is to evaluate the ability of a new type of implant (Konus Implant System®, Industrie biomediche e farmaceutiche, Italy) to isolate the internal of an implant-abutment connection from the external environment.

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 immersed 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 18% for *Porphyromonas Gingivalis* and 19% for *Tannerella Forsythia*.

Conclusion. The reported results are similar to previous work. Konus Implant System® showed bacterial leakage similar respect others implant systems (18% *Porphyromonas Gingivalis*, 19% *Tannerella Forsythia* versus 20% of Bicon® and Ankylos® systems). In spite of the limits of our study, none two-piece implant system has been demonstrated to perfectly close the gap between implant and abutment.

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



Introduction

Two-piece implant systems (TPISs) are used for oral rehabilitation both for fixed and removable prostheses (1-48).

Bacterial loading can prevent osseointegration of a TPIS during the healing phase of the surgical intervention and, if uncontrolled, can cause peri-implantitis.

The time period for bacterial contamination of a

TPIS after surgery can run from 14 days after exposure to the oral cavity to 2 or 3 months (49). Infection of implant surface of a TPIS may develop even during the surgical implant installation and proceed during the prosthetic phase. In a TPIS, the positioning of the implant-abutment connection in the supracrestal bone is important, in fact, screw loosening would allow bacterial leakage into the implant-abutment connection. It is accepted that the connection between implants and abutments in a TPIS exhibits

gaps where bacterial loading can grow and cause peri-implant tissue inflammation.

To quantify microbial colonization in the implant-abutment connection the method most often used is Real-time Polymerase Chain Reaction (RT-PCR).

Konus Implant System®

Development and engineering of the Konus Implant System® (Industrie biomediche e farmaceutiche, Italy) includes the broadest selection of prosthetic components on the today's market. All components are optimally suited and matched to the Konus Implant System® and allow for a large variety of dental treatment possibilities. The Konus Implant System® components and implant system assure for the best possible clinical and aesthetic results. The special external hex connection allows for a tight connection and allows for 360° placement of the prosthetics.

The external hex interface is the most established connection system on the market, and the preferred choice for many training institutions and top-end users. Many clinicians worldwide continue to prefer the external hex for its ease of use, widespread availability, long history of clinical success, and from mere personal preference. It is also known to be more "forgiving" in situations of impassive fit or implant divergence. Konus Implant System® has therefore continued to refine its external hex range, to provide its loyal supporters with exceptional reliability alongside proven modern features.

When completed an Konus implant/abutment connection is like a one-piece implant.

Konus Implant System® Connection

The development of external hex connections in implant dentistry has been reached and de-

scribed in literature and publications. The Konus Implant System® connects the implant and abutment and gives an optimal load dispersion of the abutment into the implant. This allows for optimal product characteristics. The connection of the Konus Implant System® is so precise, that with 3 mm diameter implants allows for optimal restorations and chewing abilities. Also the bacterial seal and micro-free movement connection assure optimal aesthetic results and avoid bone- and papilla-loss. In today's implantology the usage of TPISs is more prominent than one-piece implants. This is due to clinical and prosthetic advantages with TPISs. By usage of TPISs, the peri-implant bone height is dependent upon which implant system is used. The influence of the peri-implant bone height is dependent from the mechanical and microbiological aspects of the abutment/implant connection. Through the mechanical connection and precision of this connection, screw loosening and eventually fractures in the area of the implant/abutment connection can be prevented. The micro-movement of the implant/abutment connection and the increasing micro-gap has been studied and verified by many clinicians. Zipprich et al. demonstrated bacterial leakage by simulating chewing with an X-ray video recording (50). Implant systems with an external hex should no present significant gaps or bacterial influence. In today's clinical practice we find extreme variances and differences of implant and abutment connections.

Aim

The aim of our study is to evaluate ability of a new type of implant (Konus Implant System®, Industrie biomediche e farmaceutiche, Italy) to isolate the internal of an implant-abutment connection from the external environment.

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 shaking incubator. Four Konus Implants System® (Konus Implants®, Industrie farmaceutiche e biomediche, Italy) were used in this study. Few microliters of LB with antibiotics were put inside the implants. The implants and the abutment are screwed with a force of 35 Newton.

Few microliters of this culture were used to “contaminate” fresh LB with antibiotics contained in a micro centrifuge 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

micro centrifuge 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 Ref-Seq 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 18%

for *Porphyromonas Gingivalis* and 19% for *Tannerella Forsythia*.

The analysis revealed that in both cases (internally and externally), bacteria grew for the first 48 hours but subsequently they started to die, probably as a consequence of nutrient consump-

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	3671646	1 INSIDE	P. gingivalis	548110	14%
	T. forsythia	3482534		T. forsythia	519839	14%
2 OUTSIDE	P. gingivalis	3590985	2 INSIDE	P. gingivalis	774478	21%
	T. forsythia	5829062		T. forsythia	1631951	27%
3 OUTSIDE	P. gingivalis	5042971	3 INSIDE	P. gingivalis	1350469	26%
	T. forsythia	5014941		T. forsythia	1203405	23%
4 OUTSIDE	P. gingivalis	4023610	4 INSIDE	P. gingivalis	352157	8%
	T. forsythia	4078895		T. forsythia	339744	8%
5 OUTSIDE	P. gingivalis	2493735	5 INSIDE	P. gingivalis	789779	31%
	T. forsythia	2414858		T. forsythia	793444	32%
6 OUTSIDE	P. gingivalis	1791008	6 INSIDE	P. gingivalis	518165	28%
	T. forsythia	1755073		T. forsythia	509566	29%
7 OUTSIDE	P. gingivalis	2991545	7 INSIDE	P. gingivalis	536357	17%
	T. forsythia	3008513		T. forsythia	552056	18%
8 OUTSIDE	P. gingivalis	1976481	8 INSIDE	P. gingivalis	385105	19%
	T. forsythia	1893747		T. forsythia	369124	19%
9 OUTSIDE	P. gingivalis	2161590	9 INSIDE	P. gingivalis	856245	39%
	T. forsythia	2081358		T. forsythia	745513	35%
10 OUTSIDE	P. gingivalis	7197591	10 INSIDE	P. gingivalis	630184	8%
	T. forsythia	6960251		T. forsythia	621550	8%
11 OUTSIDE	P. gingivalis	2293864	11 INSIDE	P. gingivalis	102427	4%
	T. forsythia	2350824		T. forsythia	101412	4%
12 OUTSIDE	P. gingivalis	2047761	12 INSIDE	P. gingivalis	349551	17%
	T. forsythia	2039390		T. forsythia	290098	17%
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	3273565		PorG	599419	18%
	TanF	3409120		TanF	639808	19%

tion. Moreover, the difference between outer and inner bacteria concentration was statistically significant at each time point.

Discussion

A tight integration between abutment and implant is one of the primary objectives of a TPIS during prosthesis phase and it is of paramount importance to reduce the risk of peri-implantitis (34, 35, 37, 51-90). This can happen also in patients with syndroms (91-93, 105-109) and tumors (94-98).

Micro-cavities between implant and abutment favour bacterial leakage both from the external medium to the interior of the implant-abutment connection in a TPIS. The size of these micro cavities and gaps of a TPIS has been investigated by other Authors who tried to correlate its increase with bacterial leakage (49, 50, 99-103). The aim of this *in vitro* study was to evaluate the bacterial leakage along the implant-abutment connection by RT-PCR. The detection of implants external contamination evidences that micro cavities may be a passage to the external medium.

In a recent *in vitro* study, do Nascimento et al. showed similar bacterial leakage through the interface implant-abutment of different TPISs. Bacterial leakage through the implant-abutment connection has also been shown in other *in vitro* studies (99).

The present study demonstrated a low rate of movement of microorganisms from the implant internal region to the exterior, due to this TPIS type of connection, constructed to prevent leakage through any interface. Quirynen et al. detected higher bacterial leakage in the interior of implants, which were totally submersed in the culture medium. These Authors thus showed that bacterial leakage through the prosthetic screw also (100).

Nakazato et al., however, established that it takes only 4 hours for bacterial colonies to form on abutment surfaces (101).

While conventional cultures detect only viable bacterial cells, RT-PCR studies compiles both viable and nonviable cells, and this may be a possible explanation for the differences detected by the two methods. Mombelli et al. described limitations in bacterial cell quantification in the conventional method observing a variation of up to 24% in the bacterial loading values in one single sample, as evidence of the limited precision of the method, especially when dealing with species with high aggregation tendencies (104, 110, 111).

The limitation of this study may be the low number of microorganisms encountered in the implant interior, but new *in vivo* longitudinal studies are necessary to establish their influence in long-term clinical situations.

Conclusion

The RT-PCR has been shown to be more sensitive than the conventional bacterial culture method to detect *in vitro* contamination of dental implants. The detection of bacteria in the internal parts of this TPIS diminishes with time, suggesting reduction of bacterial viability and damage of genetic material. The RT-PCR may present advantages over the culture methods in the identification and quantification of bacteria associated with implant components and peri-implant tissues.

The reported results are similar to previous work (103). Konus Implant System® showed bacterial leakage similar respect others implant systems (18% Porphyromonas Gingivalis, 19% Tannerella Forsitya 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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