Bacterial microleakage at the abutment-implant interface – in vitro study

The potential of the implementation of additive technologies in the dentistry students’ education

Carlos Larrucea, Aparicio Conrado, Denise Olivares, Carlos Padilla, Andrea Barrera, Olga Lobos

**Streszczenie:** Introduction: In implant rehabilitation, a microspace is created at the abutment-implant interface (AII). Previous research has shown that oral microbiome can proliferate in this microspace and affect periimplant tissues, causing inflammation in periimplant tissues. Preventing microbial leakages through the AII is therefore an important goal in implantology.

Objective: To determine the presence of marginal bacterial microleakage at the AII according to the torque applied to the prosthetic implant in vitro.

Material and Methods: Twenty-five Ticare Inhex internal conical implants (MG Mozo-Grau, Valladolid, Espana) were connected to a prosthetic abutment using torques of <10, 10, 20, 30, and 30 N and then sealed. The samples were submitted to cycles of occlusal loads and thermocycling, then one sample of each group was observed by micro TC, while the rest were mounted on devices according to the bacterial leakage model with *Porphyromonas gingivalis*.

Results: Bacterial leakage was observed only in the <10 and 10 N torque samples, and the same groups presented poor abutment/implant adjustment as determined by micro-CT.

Conclusion: The different torques applied to the abutment-implant system condition the bacterial leakage at the implant interface. No microleakage was observed at 20 and 30 N.

Key words: implant, internal connection, micro CT, microleakage, torque.

**Introduction**

The use of dental implants to treat total or partially edentulous patients has become a therapeutic modality integrated in dentistry.\(^1\) Functional restoration. This can be accomplished with a careful balance of parameters such as the morphology and surface of the implant, the conditions of the receiving tissue, the surgical technique, the prosthesis design and the way in which the load is applied.\(^1\)

Currently, implant rehabilitation requires more than successful osseointegration, studies have described the need for a harmonious relationship between the levels of periimplant tissue and the already existing dentition to achieve an aesthetic and functional restoration. This can be accomplished with a careful balance of parameters such as the morphology and surface of the implant, the conditions of the receiving tissue, the surgical technique, the prosthesis design and the way in which the load is applied.\(^1\)

The exponential growth in the use of implants during the last 20 years has taken place parallel to the expansion in the field of implant manufacture. Currently there are more than 90 available types,
offering countless combinations of implant body designs, platforms, diameter, length, prosthetic connections, and state of surfaces and interfaces.6

Most current implant systems have two main components, an endosteal part (the implant) and the transmucosal connection (the abutment). When the abutment is placed in the corresponding implant, it creates a microspace, the abutment-implant interface (AII). Research has shown that the oral microbiota can proliferate in this microspace and affect all periimplant tissues, causing inflammation in the vicinity of the alveolar bone.4,5,7-9

Nowadays, it is well accepted that periimplant mucositis and periimplantitis are induced by bacterial biofilm; indeed, the presence of periimplantitis is observed in 10% to 50% of all failed implant cases after the first year of loading, where microorganisms play an important role.10 Therefore, identification of the microbiota associated with periimplantitis is crucial for understanding its pathogenesis and the bacteria that could serve as microbial biomarkers of this condition.11

Longitudinal studies of biofilm formation around dental implants have shown that bacterial colonization occurs 30 minutes after placing the implant.12 Initial colonization of the area surrounding the implant by bacteria associated with periimplantitis (Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia) is detected 2 weeks after placing the implant, and colonization by the bacteria around healthy teeth and the healthy area around the implant remains similar for a period of at least 2 years.12,13 However, the presence of periodontal pathogens in periimplant sites does not determine the loss or failure of an implant, supposing that proper oral hygiene practices are followed and periodontal therapy support exists.14

Despite the fact that no single bacteria can be identified as solely responsible for the infection of any implant system, it has been suggested that one of the key periodontal pathogens in the development of periimplantitis may be Porphyromonas gingivalis, a Gram-negative, strictly anaerobic bacteria, which is not only responsible for periodontal disease in natural dentition but is also associated with the destruction of tissue around the implant; moreover, higher rates of colony-forming units (CFU) of species such as P. gingivalis, T. forsythia, and T. denticola have also been reported in failed implants.15,16 There is also a significant relationship between the probing depth in periimplant areas and the detection of P. gingivalis.17 In another research paper, the bacterial species found in the healthy periimplant tissue was compared to those found in the diseased periimplant tissue and it was determined that the P. gingivalis was only found in the diseased cases.18

It has been observed that structural and topographical differences between the surface of implants and natural teeth can influence a unique bacterial composition that has not yet been identified.19 Thus, additional studies that recognize these concepts are needed to fully characterize the microbiota associated with periimplant disease and to develop strategies that enable their control to optimize the prevention and clinical management of periimplantitis.

When a prosthetic abutment is positioned in the implant, a microspace between the components is formed where pathogenic microorganisms can grow and establish a reservoir of bacteria, resulting in an area of inflamed soft tissue covering the abutment-implant interface.9

In vitro studies have described the potential of microbial leaks at the AII under conditions with and without load.8,20-22 Despite the fact that such in vitro studies only approach biological reality, they can be useful for understanding the dynamics of the AII, and therefore be useful for improving the design of the microspace. For example, some of these in vitro studies have shown that the design of the AII may affect the extent of microbial penetration into the inner part of the implant, implants with an external hexagon design presented the greatest microbial penetration into the AII under load and non-load conditions. This may partly explain the histological findings of in vivo studies which evaluate implants with an external hexagonal connection design, which showed increased inflammation of the connective tissue around the AII.23

Bacterial migration through the AII has also been correlated to the torque applied between the abutment and implant, micro-movements of the various components during chewing cycles and the precision of the fit between the implant and the abutment. Although the complete prevention of microbial penetration has not been demonstrated in vitro, it has been seen that internal conical connections give better results than either internal or external hexagonal connections.24,25

Whatever the abutment-implant connection used, the size of the microspace in the AII increases under load, an effect known as pumping, whe-
The aim of this study, therefore, was to determine in vitro the degree of adjustment between implant/abutment complementing visualization of the AII. However, no evidence exists to be analyzed and three-dimensional reconstructions to be made, observed radiologically by micro CT, which allows the microstructure of the microspace at the AII to be analyzed. Micro CT is of great help in determining leakage in the marginal area, which has been studied by several authors. The degree of adjustment between implant/abutment can be evaluated this variable in relation to the above-mentioned factors.

In the above context, the prevention of microbial leakage at the abutment-implant interface using as a variable the torque applied to the prosthetic components of the abutment and the implant may reach 66 lm in a vertical direction, 108 in a rotary direction and 99 lm horizontally, although the exact figures vary with the type of implant system. The tolerance of some systems may be as low as 5 lm and less than 18 rotationally.

The tolerance of some systems may be as low as 5 lm and less than 18 rotationally.

The apical zone of each implant was specially prepared with a 1 mm diameter transfixing hole following the longer axis of the implant, to compromise the internal screw where the abutment is seated, as seen in Figure 1.

Companies that design and manufacture implants have attempted to reduce such leakage by increasing the precision and stability of the articulated parts by mechanizing production techniques. The literature describes how maladjustment between the components of the abutment and the implant may reach 66 lm in a vertical direction, 108 in a rotary direction and 99 lm horizontally, although the exact figures vary with the type of implant system. The tolerance of some systems may be as low as 5 lm and less than 18 rotationally.

The importance of the position, size and geometry of the microspace at the AII on the marginal bone levels has been studied by several authors, who have demonstrated that bacterial colonization is probably the direct consequence of a poor or ineffective degree of tolerance between the implant and abutment, which increases the size of the microspace. The precision of the adjustment may affect the penetration of bacteria, thus establishing a microbiological reservoir. Another point to bear in mind is the seal proposed by the manufacturer, to
obtain the best possible fit in the system geometry. Studies have evaluated different torque values applied in the connection of the prosthetic abutment and implant, and concluded that a low value produces a poor connection and increases bacterial microleakage.\textsuperscript{20,24}

In the study described by Baggi et al.,\textsuperscript{20} although the abutments were connected to the implants with the recommended torque, the geometry of some systems still permitted the passage of microorganisms. This is probably due to the different degrees of tolerance and different interface geometries that different implant systems and brands allow.

In an \textit{in vitro} study with methylene blue dye, Larrucea et al.\textsuperscript{24} observed that internal conical connection showed lower levels of microleakage than those with an external connection, regardless of the torque applied to join the abutment and implant. They would therefore be less likely to
TABLE 1: Bacterial leakage by groups and days of incubation using double chamber device.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Subgroup</th>
<th>Incubation (days)</th>
<th>Number of samples with positive leakage</th>
<th>% of samples with positive leakage per subgroup (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>A</td>
<td>1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Group II</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Group III</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Group IV</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Group V</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

**Fig. 6**

**Fig 6:** Interface of the abutment of the sample from Group I (10 Ncm torque,) visualization window 4.53 3 2.63 mm. I: Transversal view. II: Frontal view. III: Close-up of frontal view.

**Fig. 7**

**Fig 7:** Interface of the abutment of the sample from Group II (20 Ncm torque), Viewing window 4.53 3 2.63 mm. I: transversal view. II: Frontal View. III: Close-up of frontal view.
In this investigation, the prevention of microleakage at the AII is an important challenge in the reconstruction of two-piece implant systems to minimize inflammatory reactions and maximize the stability of the bone around the implant.\(^8\) The degree of adjustment between implant/abutment can be observed radiologically by micro CT, which allows the microstructure to be analyzed and three-dimensional reconstructions to be made, complementing visualization of the AII.\(^{28}\) However, no evidence exists to evaluate this variable in relation to the above-mentioned factors.

In the above context, the prevention of microbial leakage at the AII is an important challenge for...
the design and manufacture of two pieces implants, that permit minimizing inflammatory reactions and maximizing the stability of the bone surrounding the implant."}

The aim of this study, therefore, was to determine in vitro the presence of marginal bacterial microleakage at the abutment-implant interface using as a variable the torque applied to the prosthetic abutment.

Methodology

The experimental study followed a qualitative approach, with a sample of 25 Ticare Inhex internal implants with conical connection (10-mm length and 3.75-mm platform from MG Mozo-Grau, Valladolid, Spain). The apical zone of each implant was specially prepared with a 1 mm diameter transfixing hole following the longer axis of the implant, to the internal screw where the abutment is seated, as seen in figure 1. The rest of the implant geometry, including the coronal area, was manufactured following normal standard procedures for this brand. The implants were directly supplied by the manufacturer with a premounted straight hexagonal abutment for cementing with Ticare Inhex MTA mount function (MG Mozo-Grau InHex MTA, Valladolid, Spain).

Each implant with its respective abutment was mounted on a standard cylinder, 2.8-cm high and 2.2-cm diameter, made of transparent self-curing acrylic prepared as indicated by the manufacturer (one third monomer, two thirds polymer), in which the implant was inserted with ISO 14801:2008.

The total sample was randomly divided into five experimental groups, and different torque was applied to each group:
- Group I (n=5): Prosthetic abutment attached to the implant with 10 N torque applied.
- Group II (n=5): Prosthetic abutment attached to the implant with 20 N torque applied.
- Group III (n=5): Prosthetic abutment attached to the implant with 30 N torque applied.
- Group IV (n=5): Prosthetic abutment attached to the implant with 30 N torque applied and interface sealed with cyanoacrylate adhesive (Fenedur, Uruguay).
- Group V (n=5): Prosthetic abutment joined to the implant with <10 N torque applied.

The whole procedure was carried out by one investigator using a standard torque wrench from Mozo-Grau (Valladolid, Spain). The access to the abutment screw was sealed with Teflon and Fermin (Detax Dental, Ettlingen, Germany). The samples were submitted to perpendicular loading cycles (in relation the major axis of the acrylic cylinder) over the inclined implant abutment, a total of 2000 cycles of 10 kg every 0.5 seconds being applied (Fig. 3).

The 25 samples were submitted to 300 thermal cycles, each cycle consisting in 5 seconds immersion with the water temperature alternating from 58 C to 508 C.

After the loading and the thermic cycles, the samples were extracted from the acrylic cylinder, and one
sample of each group (chosen randomly according to www.random.org) was fixed on a Teflon platform (Fig. 4) and scanned with a micro-CT (Nikon XT H 225, Tokyo Japan). The x-ray parameters used were 140 kV-tube voltage and 90-lA current. 720 images were taken and 4 squares per projection were used for the 3D volumetric reconstruction.

The remaining samples (n 520) were mounted on devices in accordance with the bacterial leakage model29 modified in the Laboratorio de Investigacion Microbiologica de la Universidad de Talca (Universidad de Talca, Talca, Chile). Each device was composed of two chambers connected only by the implant (Fig. 5); the upper chamber was a 1.5 ml Eppendorf tube (Biologix Research Company) with hermetic cap, and the lower chamber was a 10 ml-glass tube with a plastic cap. The Eppendorf tube was cut open at the end and the implant was screwed in as far as the first microthread, and sealed with fluid resin (Filtek flow, 3M ESPE Dental Products St. Paul, Minnesota). The abutment extending from the tube was in contact with the lower glass chamber.

The Eppendorf tube was fixed to the plastic cap of the glass flask by cyanoacrylate adhesive (Fendur, Uruguay), adding a 30G calibre Luer type needle (Cranberry, China), which allowed the passage of 5ml sterile thioglycolate broth (Becton, Dickinson and Co. 7 Loventon Circle, Sparks, MD), and the release of gases. The device was allowed to set for 24 hours and was then sterilized with ethylene oxide.

In a sterile atmosphere, in a Biosecurity Class II cabinet (NuAireTM, Plymouth) the upper chamber was loaded with a thioglycolated semisolid medium of (1.4 ml thioglycolate broth with 0.8% agar agar) (Becton, Dickinson and Co.). Then, the lower chamber was loaded with a culture of 50 ll P. gingivalis in thioglycolate broth.

P. gingivalis was isolated from clinical origin and identified by molecular biology following the method described by Park et al.30 The study began with an inoculum concentration similar to 0.5 Mc Farland (1.5 3 108 cfu/ml) (Probac do Brasil, Sao Paulo, Brasil), with the implant abutments immersed in the culture medium with P. gingivalis. The samples from groups I, II, III, IV, and V were then divided into two subgroups, A and B, the first (A) left to set and the second (B) submitted to constant stirring at 150 rpm. Both subgroups were incubated for 15 days at 378 C under anerobiosis in a culture oven (VWR, Sheldon Manufacturing, Mod. 1510E-2). The device was inspected daily by transversal light. Any development of bacteria in the lower chamber was assessed from the turbidity of the thioglycolate broth, while turbidity in the upper chamber was taken as being indicative of microleakage. All assays with both subgroups were carried out in duplicate.

After determining positivity in the sample, the device was separated from the non-contaminated samples. The number of days that took for leakage to occur and the group to which the sample belonged were recorded. A sample was taken from the devices in which growth was evident and cultured in hemin-menadione blood agar, which was incubated anaerobically at 378 C for 7-14 days to confirm the development of P. gingivalis, whose identity was confirmed by molecular biology, as described above.

Results

The results of the duplicate study after processing the 20 samples in the bacterial leakage model are shown in Table 1. In the samples of Group I and Group V, bacterial leakage occurred from the lower chamber to the upper chamber of the device. All the samples from Group I (n54) and Group V (n54), with and without shaking, showed leakage from day 3 onwards. Groups II, III, and IV showed no leakage at any time during the experiment.

Observation by Micro CT of the frontal and horizontal sections (Fig. 6) showed a slight connecting interface in the sample of Group I, to which a torque of 10 N had been applied. There was also a slight asymmetry between the opposite walls in the close-up of the frontal view. The image was related with the microbiological results, whereby the poor fit between implant and abutment was responsible for the bacterial leakage from the lower to upper chamber of the device.

Observation of the samples from group II (Fig. 7), and from Groups III and IV (Fig. 8 and 9, respectively), by micro-CT did not suggest the presence of any misadjustment in the implant-abutment interface in any of the viewing angles or close-up.

Group V, (Fig. 10) showed a clear connecting interface. The frontal close-up shows the asymmetry between the opposite walls. The image is related with the microbiological results, and the lack of adjustment between implant and abutment led to the leakage.

Discussion

The results of this investigation present no evidence of bacterial leakage in the internal co-
technical connection implants studied when a torque of 20 or 30 N was applied. This reflects the recommendations of the manufacturer (30 N), as bacterial leakage was observed in the AII in samples with a torque equal to 10 N, apart from an evident lack of adjustment between the implant and abutment, as observed by micro-CT.

Bacterial colonization of the AII depends on factors such as the precise adjustment between the implant components, and the torque between these components and the load when implants are functioning.\cite{31}

This study simulated mechanical loads to which the implant abutment system is exposed (compression and torsion) by means of occlusal load cycles and thermocycling, following the directives of ISO 1480. These same conditions have been simulated in other studies.\cite{7,20,22,24,32}

It was seen that bacterial leakage at the interface is influenced by constant shaking in the system of chambers used in this experiment, as demonstrated by Koutouzis et al.,\cite{22} who used Escherichia coli. However, in this investigation was decided to use \textit{P. gingivalis}, as it is commonly isolated in zones with mucositis and peri-implantitis.\cite{33}

Other authors, such as Baggi et al.,\cite{20} demonstrated that, although the abutments were connected to implants using the recommended torque, the geometry of some systems, including internal conical connections, permitted bacteria to enter and exit. This observation is not related with our findings here since, when the torque recommended by the manufacturer was applied (30 N), there was no bacterial leakage or deformation of the abutment geometry within the implant, as corroborated by the micro-CT scans. However, the results should be treated with caution as the simulated system is more passive than it would be \textit{in vivo}.

When the force of the union between implant and abutment is less than recommended there is a clear lack of connection in the system, as reflected by the presence of bacterial leakage, a situation corroborated by authors like Baggi et al.\cite{20} and Larrucea et al.\cite{24} Indeed, the latter demonstrated using methylene blue that the AII was permeable when the torque applied to the internal connection was only 20 N.

Conclusions

According to the results obtained in this study, it can be concluded that:

- The torque applied to the implant-abutment system conditions the permeability of bacteria to the interface, which is evident at a torque of less than 20 N, and with zero permeability at torques of 20 and 30 N.
- The abutment-implant coupling is also dependent on the torque applied to the system, and results in maladjustment at values of 10 N or less.
- Micro-CT points to an evident lack of adjustment between implant and abutment in the group of <10 and 10 N torque but not when 20 or 30 N was applied.

Acknowledgment

The authors acknowledge Mozo-Grau Ticare Dental Implant Company for their contribution in the development of this study.

Conflict of interest

No conflict of interest

Orcid

Carlos Larueca DDS, MSc http://orcid.org/0000-0002-7665-2860

References:


authors:

Carlos Larrucea DDS MSc – Denise Olivares DDS
Postgrado de Rehabilitacion Oral
Universidad de Talca, Talca, Chile

Aparicio Conrado PhD – Minnesota Dental Research Center for Biomaterials and Biomechanics University of Minnesota
Minneapolis, Minnesota

Carlos Padilla MSc – Andrea Barrera MSc, Olga Lobos MSc
Departamento de Microbiologia,
Universidad de Talca, Talca, Chile

Contact:

Prof. dr Carlos Larrucea
Postgrado de Rehabilitacion Oral Universidade de Talca,
Talca, Chile
E-mail: larrucea@utalca.cl

E-mail: larrucea@utalca.cl

Talca, Chile

Postgrado de Rehabilitacion Oral Universidad de Talca,
Talca, Chile

 implicates

Postgrado de Rehabilitacion Oral Universidad de Talca,
Talca, Chile

Talca, Chile

Contact:

Prof. dr Carlos Larrucea
Postgrado de Rehabilitacion Oral Universidad de Talca,
Talca, Chile
E-mail: larrucea@utalca.cl

 implicates

Postgrado de Rehabilitacion Oral Universidad de Talca,
Talca, Chile

Talca, Chile

Contact:

Prof. dr Carlos Larrucea
Postgrado de Rehabilitacion Oral Universidad de Talca,
Talca, Chile
E-mail: larrucea@utalca.cl

 implicates