Evaluation of resistance against bacterial microleakage of a new conical implant-abutment connection versus conventional connections: an in vitro study

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SUMMARY
The aim of the present in vitro study was to evaluate bacterial microleakage from inside to outside the implant-abutment assembly in a new design of internal conical connection compared to eight different internal connections. The design of this connection should prevent or limit microbiologic leakage into the surrounding implant tissue, that could contribute to infections without bone loss (mucositis) or with bone loss (peri-implantitis). In order to investigate bacterial microleakage, the inner part of each system was inoculated with an Escherichia coli suspension. Eight different groups were considered; each group was composed of 10 dental implants, for a total of 80 implants. Groups 1-7 were considered controls, while group 8 was the test connection (an internal connection characterized by a double taper principle). Results showed that in control implants (Group 1 to 7), little microleakage was observed after the first 6 hours (500 CFU/µl) and, after 24 hours of incubation, they showed a significant bacterial contamination in all samples (>100,000 CFU/µl). In group 8 (test connection) no contamination was found in the first 6 hours, with 7 out of 10 implants showing no contamination even after 96 hours. Statistically significant differences were found between Group 8 and the other groups (p<0.05), whereas no significant differences were found among implants of the control groups (from group 1 to 7). Within the limits of the present study, the new connection studied presented significantly less microleakage at 96 h in comparison with the other control internal connections.

KEY WORDS: Dental implant, Connection, Abutment, Internal conical, Taper connection, Bacterial leakage.

INTRODUCTION
Today dental implants are a common choice for rehabilitation of partial or completely edentulous patients and a successful implant therapy demands a balance between biological and mechanical factors (Callen et al., 1998). Poor plaque control is considered a biological risk factor for implant disease (Callen et al., 1998).

In fact microbial accumulation around dental implants may cause infections of peri-implant tissues known as peri-implant mucositis when limited to soft tissues and peri-implantitis when bone loss occurs around the implant and may lead to implant failure (Callen et al., 1998). Additionally, when an abutment is used on an implant, bacteria can colonize the inside of the abutment (Gross et al., 1999). All the evidence suggests that the first colonization happens during surgery or after placement of the abutment (Broggini et al., 2003). Many studies have revealed bacteria both outside and between the implant components, and also within them (Aloise et al., 2010; Broggini...
Microleakage at the abutment-implant interface can occur and according to Broggiini et al., an increase in inflammatory cells can be found in the peri-implant soft tissues at the level or slightly coronal to the implant-abutment junction (Broggiini et al., 2003). The bacteria found in this space can be both anaerobic and facultative anaerobic depending on the features of this microhabitat. It is important to remember that individuals who have been treated for periodontal disease have a higher risk of peri-implantitis (Sgolastra et al., 2015).

Pathogens in the oral cavity must be reduced by proper plaque control before each surgical phase (Sgolastra et al., 2015). The design of the fixture-abutment interface may have an impact on the amount of microbial leakage between the two parts. Many types of abutment-implant connections are present on the market, the most common are internal, where a part of the abutment is inserted in the body of the implant, and external, where the abutment is placed above the implant. Research led by Larrucea Verdugo et al., revealed that Morse taper connection implants show lower levels of microleakage than external connection implants (Larrucea Vedugo et al., 2014). Furthermore, the internal conical implant-abutment connection has been considered mechanically more stable and tighter than flat-to-flat or tube-in tube connections (Harder et al., 2010). Based on these findings, a new type of conical internal connection with a tight seal between the abutment and the internal wall of the implant was designed. The new design should offer greater stability to the implant-abutment complex giving a prosthetic advantage to patient rehabilitation. In addition, it should be able to prevent or limit microbiologic leakage into the surrounding implant tissue, that could contribute to infections without bone loss (mucositis) or with bone loss (peri-implantitis). Peri-implantitis can ultimately lead to loss of the implant itself.

The aim of the present in vitro study was to evaluate bacterial microleakage from the inside to the outside of the implant-abutment assembly in eight different internal connections, testing the new design of internal conical connection.

### Materials and Methods

In this in vitro study, 8 different groups were scheduled. Each group was composed of 10 dental implants, for a total of 80 implants. The type of connections and dental implant groups are listed in table 1. Groups 1-7 were considered controls, while group 8 was the test connection (DAT connection (Double Action Tight) used for CSR-DAT implants, Sweden & Martina, Padua, Italy). This is an internal connection characterized by a double taper principle. The first taper is an internal cone that supports and closes the prosthesis and is combined with an internal hexagon. This is used for the implant screwing and the prosthesis repositioning. The second taper is an interaction surface between the prosthetic abutment and the head of the tightening screw, which is conical. The angles and working depths in the interaction between implant, abutment and screw should enhance the mechanical resistance and minimize the process of bacterial leakage through the double seal achieved in the interaction of the two conical surfaces (Figure 1-3).

TABLE 1 - Groups, number of implants and type of connection

<table>
<thead>
<tr>
<th>Group</th>
<th># Implants</th>
<th>Type of connection</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Morse Locking Taper (Bicon, Boston, MA, USA)</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Conical connection (Conical Seal Design, Astra Tech, Dentsply Friadent, Mannheim, Germany)</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Internal hexagon (Free Lock, Winsix, Biosafin, Ancona, Italy)</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>Conical connection (TTc, Winsix, Biosafin, Ancona, Italy)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>Morse Locking Taper (TTcm, Winsix, Biosafin, Ancona, Italy)</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>Internal hexagon (Premium, Sweden e Martina, Italy)</td>
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<tr>
<td>7</td>
<td>10</td>
<td>Internal hexagon (CollexShelta, Sweden e Martina, Italy)</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>Internal connection, double taper (DAT, Sweden e Martina, Padua, Italy)</td>
</tr>
</tbody>
</table>

Sterilization of implants and abutments before testing

In order to avoid external contamination, all implants, abutments and instruments to be
used to handle the test materials were sterilized for at least 4h at 250°C in a dry oven. All the experimental procedures were performed in sterile conditions in a proper microbiological hood under vertical laminar flow.

**Test setup**
All implant fixtures were fixed on dedicated holders under sterile conditions.

**Preparation of the implants**
Before the final connection of the test abutments to the corresponding implants, $10^5$ colony forming units (CFU) of *Escherichia coli* (Reference strain ATCC 25922) in 3 µl of Luria-Bertani (LB) broth (Sigma-Aldrich) were carefully pipetted to the deepest point of the internal lumen of each implant (Figure 4). A single implant of each type was inoculated only with 3 µl of LB and was used as negative control.

**FIGURE 1 - DAT connection (Double Action Tight) used for CSR-DAT implants is an internal connection characterized by a double taper principle. Blue arrow: implant abutment, red arrow: implant fixture.**

**FIGURE 2 - The angles and working depths in the interaction between implant, abutment and screw help to enhance the mechanical resistance and minimize the process of bacterial leakage through the double seal achieved in the interaction of the two conical surfaces.**

**FIGURE 3 - Section of the connection, light microscopy view, 20x magnification.**
*Escherichia coli* is a Gram-negative, facultative anaerobe, rod-shaped bacterium, whose size ranges from 1.1 to 1.5 µm in diameter and from 2 to 6 µm long. A McFarland’s 0.5% bacterial suspension (corresponding to $1.5 \times 10^8$ CFU/mL) was prepared in saline starting from colonies grown on MacConkey agar (BioMérieux, Marcy-l’Étoile, France). The solution was therefore used to contaminate the inner part of the implants following an already described protocol (Jansen V.K. *et al*., 1997; Assenza *et al*., 2012; Rismanchian *et al*., 2012).

After this preparation, the abutments were then connected using a calibrated torque controller following the manufacturer’s instructions and being extremely careful not to touch the outer or inner surfaces of the implants. After fixing the abutment, all implants were carefully investigated to detect possible over-pressing of pipetted bacterial culture. The outer surfaces of the implants were then cleaned with sterile alcohol gauzes, and abundantly rinsed with sterile saline (Figure 5). Each implant was sequentially passaged in two 1.5 ml LPS-free Eppendorf tubes (Eppendorf S.r.l. Milano) (Sterility control tubes A and B), and then finally immersed in a 500 µl micro tube (Test tube) containing approximately 250 µl of LB (volume needed to cover completely the implant-abutment connection) (Figure 6). All tubes, including sterility controls, were then incubated at 37°C.

**Growth evaluation**

Five µl of each supernatant were collected from the test tubes using a single-channel pipette.
at different time-points (1 h, 3 h, 6 h, 16 h, 24 h, 48 h, 72 h and 96 h) and spread-plated on LB agar plates for quantification. After sample collection, an equal volume of sterile LB broth was pipetted to each test tube. Sterility control tubes were also checked for lack of growth, in order to confirm that possible growth in test tubes was due to microleakages from the implant-abutment connections. In particular, the broths were clear and showed no bacterial cloudiness (turbidity), proving an absence of external contamination of implants. The implants were then removed from the abutments and the bacteria in the internal lumen checked for vitality. An independent examiner unaware of the type of connection examined, evaluated bacterial growth.

Statistics
The difference in contamination between groups were analysed by Dunn’s multiple comparisons test for independent samples (p<0.05 was considered the threshold for statistical significance).

RESULTS
In control implants (Groups 1 to 7), small microbial leakage was observed after the first 6 hours (500 CFU/µl), becoming significant after 24 hours of incubation with a number >100,000 CFU/µl in suspension samples (Table 2, Figures 7 and 8). In group 8 (test connection) no contamination was found in the first 6 hours, while 7 of 10 implants showed no contamination at 96h time-point; 2 implants resulted contaminated at 24h with a low quantity of bacterial colonies (about 300 CFU/µl) and 1 implant at 48h. Statistically significant differences were found between Group 8 and the other groups (p<0.05), whereas no significant differences were found between implants of the control groups (p>0.05) (groups 1 to 7).

DISCUSSION
Differences in implant design may affect the potential risk for colonization of oral microorganisms in the fixture-abutment interface microgap (Koutouzis et al., 2011). The present study aimed to evaluate a new internal conical connection that should reduce bacterial infiltration by constructing a physically tight connection with a high level of precision in the sub-micrometre range. The control connections were chosen because are among the most common present on the market and among the most studied. The results from the new connection group showed that only three of the connections tested had a negligible bacterial penetration at 96 h down to the threaded part of the fixture-abutment junction under in vitro conditions, the seven remaining showed absolute no infiltration. The implant-abutment connection is usually situated under the soft tissue of the patients, sometimes very near to the bone. Since the

![FIGURE 7 - System in nutrient solution. Left: turbidity of the broth as a sign of bacterial leakage. Right: no contamination.](image-url)

<table>
<thead>
<tr>
<th>Number of contaminated implants at different time points</th>
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<tr>
<td>Group</td>
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abutment protrudes into the oral cavity, the absence of bacterial infiltration can be a major aspect in preventing the initiation of infection in the peri-implant tissues. Even if clinical studies on this aspect are still lacking, the possibility of avoiding the passage of pathogenic bacteria should be considered important, especially in patients with an history of periodontal disease. Limitations of this in vitro study were the use of only one bacterium, a limited follow-up, the testing of a static condition, and the use of only one torque force. In addition, it would be important to investigate which factors could affect the permeability of some of the connection. The bacterium used was the *Escherichia coli*, a gram-negative, motile, and facultative anaerobic bacterium measuring 1.1 to 1.5 μm in diameter and 2 to 6 μm in length. It is an opportunistic human pathogen occasionally associated with implant failure (Renvert et al., 2015) already used in microbial leakage dental implant studies (Jansen et al., 1997; Koutouzis et al., 2011; Silva-Neto et al., 2012; Jaworski et al., 2012). One study (Dibart et al., 2005) using a Morse cone-connection implant found no bacteria in the nutrient broth or in any of the implant wells at 72 h. A 2% bacterial agar mix was used. The same type of connection showed bacterial penetration in our study. No contaminated samples were found at 28 days using a cemented connection, while with a conical connection 1 out of 10 and with a tri-lobed one 6 out of 10 showed contamination (Assenza et al., 2012). *Pseudomonas aeruginosa* and *A. actinomyctemcomitans* were used (Assenza et al., 2012).

Harder et al. demonstrated that an internal conical implant-abutment connection is not tight enough to prevent endotoxin (lipopolysaccharides from *Salmonella enterica*) penetration. Indeed, while only 1 of the 16 implants was not contaminated after 168 hours (Harder et al., 2010), the other implants used were contaminated after 72 hours. Similar results were reported after inoculation of *Staphylococcus aureus* in another study which found a microleakage of 77.7% in morse taper implants and 100% in internal hexagon connections after 7 days (Teixeira et al., 2011). Aloise et al., also demonstrated in vitro the presence of contamination after two days in implants with internal conical connection and a morse taper connection, using *S. sanguinis* (Aloise et al., 2010). A longer follow up was not considered in this preliminary study since significant differences were found in comparison with the other control connections already at 24 hours. Longer...
follow-up will be planned for testing the new connection alone.

Multifactorial conditions influence the potential colonization of the microgaps, such as: the precision fit between the implant components, the torque forces used to connect the components and the loading forces when the implants are in function (Tesmer et al., 2009). Using an in vitro dynamic-loading model to assess the potential risk for invasion of oral microorganisms into the fixture-abutment interface microgap Koutouzis et al. showed that 12 out of 14 implants with internal conical connection were contaminated by Escherichia coli after 24 hours (Koutouzis et al., 2011). This last aspect was not investigated in the present study and should be tested in future in a dynamic-loading model.

The torque used in this study followed the recommendations of the manufacturer not to interfere with the results. One study showed that the degree of leakage found depended on the closing torque and there was an inverse correlation between the degree of closing torque and the severity of the leakage, the higher the torque intensity, the less leakage was observed (Gross et al., 1999). However, another study (Silva-Neto et al., 2012) found that the tightening torque of external hexagon connections did not affect the microleakage of Escherichia coli after 24 hours.

Additional studies are necessary to better understand the stability of this new type of internal connection for a longer period of time, with different bacteria or metabolites, which factors could affect the permeability of some of the new connections and its behaviour in mastication function. Besides the connection should be tested in clinical condition on patients.

Within the limits of the present in vitro study, the new connection studied presented significant less bacterial microleakage at 96 h in comparison with the other control internal connections and this could present a clinical advantage in the prevention of peri-implant tissue infection.

REFERENCES


