



# Microbial Leakage at Morse Taper Conometric Prosthetic Connection: An *In Vitro* Investigation

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The use of osseointegrated implants is a successful and widely used treatment option for both partially and totally edentulous patients. Dental implants can be used for supporting removable or fixed reconstructions, namely implant-supported fixed dental prostheses (IFDPs).<sup>1</sup> Although good long-term survival of IFDP was demonstrated, complications may be frequent.<sup>2–5</sup> Typically, 2 main types of complications have to be faced by clinicians: mechanical, such as screw loosening and fractures and biological, such as periimplant disease.<sup>6,7</sup>

Interfaces between the endosseous fixture and prosthetic components (such as abutments or attachments) are present in implant systems; often microgaps may be present at such level.<sup>8</sup> The presence of excessive misfits between components is a critical

**Objective:** To evaluate *in vitro* the sealing capability at the prosthetic connection interface of 2 conometric systems.

**Materials and Methods:** Two conometric systems with the same design and different material were used, for a total of 24 samples. Each sample was assembled by a tapered abutment and respective coping. In group A, the copings were made of gold, whereas in group B they were made of PEEK. Three  $\mu\text{L}$  of mix bacterial suspension (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum* species) was inoculated into the abutment screw hole, and the coping was inserted on the abutment. Samples were immersed into culture tubes and incubated for 24, 48, and 72 hours into anaerobic conditions. Visual

evaluation of turbidity was performed at each time point. Qualitative-quantitative assessment using real-time polymerase chain reaction was performed at 72 hours. Any difference between the groups was checked by means of Fisher exact test.

**Results:** Microbial leakage occurred in both groups, and there was no statistically significant difference between groups. Microbial concentration resulted in a presence inferior to  $1 \times 10^2$  copies/ $\mu\text{L}$  in all positive assemblies.

**Conclusions:** Because of the low bacterial count, it can be concluded that a minimal bacterial infiltration may be allowed by conometric interfaces for prosthetic connection. (*Implant Dent* 2017;26:756–761)

**Key Words:** dental implant, bacterial microleakage, bacterial count, Morse taper conometric system

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condition because of mechanical factors such as unfavorable stress loading and prosthetic loss of retention.<sup>9</sup> From a biological point of view, gaps have to be avoided because they could act as a microbiological reservoir favoring plaque formation.<sup>12–14</sup> Inflammatory cell content in the periimplant tissue may be triggered by bacterial presence.<sup>10</sup> Therefore, microbial accumulation leads to inflammation and infection. Bone loss and lately the breakdown of osseointegration may

occur.<sup>14–16</sup> These assumptions are confirmed by clinical data, which have shown that bacterial presence is associated with clinical conditions, such as mucositis<sup>17</sup> and implant loss.<sup>18,19</sup>

*In vivo* bacterial colonization of Brånemark system implant inner surfaces<sup>20,21</sup> suggested that microgaps between implant components are vulnerable to microbial contamination. Fluid leakage through different implant system interfaces was investigated by several *in vitro* studies.<sup>22</sup> Bacterial

leakage was deeply investigated at implant-abutment interface of two-piece implant systems.<sup>9,22–32</sup> Despite the fact that contamination of the implant components in the oral cavity seems to be inevitable, better results were exhibited by some implant-abutment design. In particular better fit, stability and seal performance may be provided by Morse taper conical abutment connection.<sup>33</sup>

Otherwise, few data are actually available on bacterial leakage at abutment to prosthesis connection level. The retention of prosthetic connection for IFDP restorations is commonly provided by means of screws or cement. Recently, the Morse taper conometric system was proposed for fixed prosthetic connection. Such system is composed by a tapered coping fixed to the prosthesis and inserted in a tapered abutment. If a proper insertion force is applied, this system is capable to provide a fixed connection.<sup>34</sup> Encouraging clinical results were reported on the Morse taper conometric system used as implant-supported complete fixed prostheses.<sup>35</sup> However, the microbiological seal performance of such a type of connection was never tested.

The aim of the present study was to evaluate with an *in vitro* model the sealing capability along the prosthetic connection interface using 2 Morse taper conometric systems.

## MATERIALS AND METHODS

Bacterial culture was performed according to ATCC (American Type Culture Collection) Bacterial Culture Guide. Three of the main bacterial strains involved in the onset and in the progression of periodontal disease/periimplantitis were used: *Aggregatibacter actinomycetemcomitans* (ATCC 33384), *Fusobacterium nucleatum* species (ATCC 10935), and *Porphyromonas gingivalis* (ATCC 33277), respectively. Bacterial pellets were rehydrated, and bacterial suspensions at 37°C in anaerobic atmosphere for 48 hours were obtained (Fig. 1).

Sample size was adopted according to previous data available on such study design.<sup>22,23,29,36</sup> Two conometric



**Fig. 1.** Anaerobic atmosphere was recreated into the incubator. ATCC guidelines were followed for growing bacterial culture.

implant systems with the same design but different material were used, for a total of 24 samples (DENTSPLY Implants Friadent, Mannheim, Germany and Leone Implants, Firenze, Italy, respectively). All test components and instruments were previously sterilized, and all procedures were aseptically conducted to prevent microbiological contamination.

In the A test group, each of 12 experimental system samples were composed of a 3.5 × 13-mm internal conical connection implant (ANKYLOS, DENTSPLY Implants Friadent, Mannheim, Germany); a tapered ANKYLOS SynCone C Abutment 1.5 straight 5° titanium abutment (DENTSPLY Implants Friadent, Mannheim, Germany) and an ANKYLOS Taper Cap Degulor gold coping (DENTSPLY Implants, Mannheim, Germany). Then, the abutment was screwed to the implant using a precalibrated 15 N·cm manual torque wrench. Implant to abutment connection consisted in a tapered internal interface.

In B test group, each of 12 experimental samples were composed of a Morse taper internal connection implant (Leone Implants, Firenze, Italy); a 5° tapered titanium abutment; and a 5° PEEK coping (Leone Implants, Firenze, Italy). Implant to abutment connection consisted in a self-locking tapered interface, so that the abutment chamber did not present a screw hole.

In 10 samples from group A and 10 samples from group B, a total of 3 μL

of mix bacterial suspension (1 μL of *A. actinomycetemcomitans*, 1 μL of *P. gingivalis*, and 1 μL of *F. nucleatum* species) was inoculated into the abutment, under sterile conditions using a calibrated micropipette. Subsequently, the coping was inserted on the abutment and an insertion force of 50 N was applied to activate the system. A calibrated dynamometric electronic press machine was used for the forces application (Leone Instruments, Firenze, Italy).

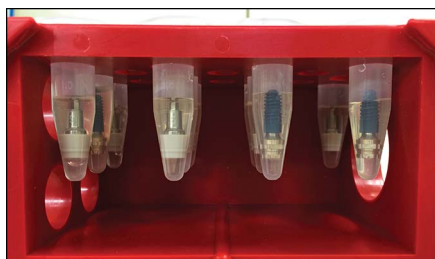
The sample was then immersed individually into culture tubes containing 0.5 mL of Trypticase Soy broth and incubated for 24, 48, and 72 hours into anaerobic conditions (Fig. 2).

One negative and 1 positive control assemblies were also assessed in each group. Group A and group B negative controls were submerged individually into 0.5 mL of Trypticase Soy broth without bacterial inoculation. Differently, group A- and group B-positive controls were submerged individually into 0.5 mL of Trypticase Soy broth with the addition of 3 μL of mix bacterial broth. Incubation was performed as in both A and B test groups.

Bacterial culture quantity was preliminary defined to avoid the bacterial suspension leakage in the culture medium and then obtain a false positive result.



**Fig. 2.** After bacterial inoculation into the abutment and coping application, samples were immersed individually into culture tubes containing 0.5 mL of Trypticase Soy broth and incubated into anaerobic condition.



**Fig. 3.** Culture tubes containing samples from group A and group B were evaluated for visual turbidity at 24, 48, and 72 hours. Positive results were recorded.

At each time point, the presence of turbidity was assessed by visual evaluation and recorded (Fig. 3). Moreover, a broth content qualitative and quantitative assessment was performed using real-time polymerase chain reaction (PCR) at 72 hours. Therefore, the presence of the 3 different bacterial species and the respective concentration were evaluated. Results were confirmed by horizontal electrophoresis performed on horizontal

agarose gels. Consequently, the amplicon obtained in PCR and the expected outcome for each of the 3 bacteria were verified.

Because of the sample size dimension, Fisher exact test was performed to check any significant difference between A and B groups. The level of significance was set at 5%.

## RESULTS

Results of samples at each time point are depicted in Table 1 and Figure 4. Negative bacterial proliferation both from visual evaluation and PCR was shown in both negative controls at each time point. Differently, medium turbidity was found in both positive controls only at 48 and 72 hours. The microbial proliferation was confirmed by real-time PCR and horizontal electrophoresis. The presence of the 3 bacterial strains in an amount of  $4.23 \times 10^5$  and  $3.45 \times 10^5$  copies/ $\mu\text{L}$  for groups A and B, respectively, was confirmed by real-time PCR results.

No positive visual turbidity evaluation was found at 24 hours. At 48 hours, visual turbidity assessment presented positive results for 1 of 10 in group A and 2 of 10 in group B assemblies, respectively. Similarly, at 72 hours, visual turbidity assessment presented positive results for 1 of 10 in group A and 3 of 10 in group B assemblies, respectively.

Real-time PCR showed positive microbial proliferation in 10 of 20 total assemblies; 4 of 10 in group A and 6 of 10 in group B, respectively. Microbial concentration of less than  $1 \times 10^2$  copies/ $\mu\text{L}$  resulted at real-time PCR in all positive assemblies. Quantitative data are provided in Table 1.

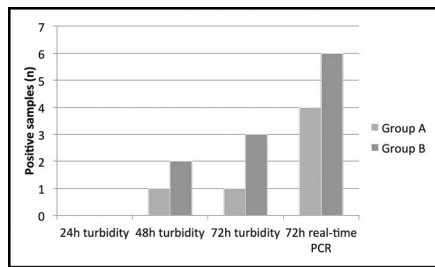
The presence of the selected bacteria in all samples was confirmed by horizontal electrophoresis. No significant differences between groups both for visual assessment at 48 hours ( $P = 0.500$ ), 72 hours ( $P = 0.291$ ), and real-time PCR ( $P = 0.328$ ) were calculated.

**Table 1.** Results From Turbidity Evaluation, Real-Time PCR, and Bacterial Count for Each Assembly are Depicted. Results are Shown as Positive (+) or Negative (–)

Specimen	Group	24 h Turbidity	48 h Turbidity	72 h Turbidity	72 h Real-Time PCR	72 h Bacterial Concentration, Copies/ $\mu\text{L}$
1	A negative control	–	–	–	–	0
2	A positive control	–	+	+	+	$4.23 \times 10^5$
3	A	–	–	–	+	$<1 \times 10^2$
4	A	–	–	–	–	
5	A	–	–	–	–	
6	A	–	+	+	+	$<1 \times 10^2$
7	A	–	–	–	–	
8	A	–	–	–	–	
9	A	–	–	–	–	
10	A	–	–	–	+	$<1 \times 10^2$
11	A	–	–	–	+	$<1 \times 10^2$
12	A	–	–	–	–	
13	B negative control	–	–	–	–	
14	B positive control	–	+	+	+	$3.45 \times 10^5$
15	B	–	–	+	+	$<1 \times 10^2$
16	B	–	+	+	+	$<1 \times 10^2$
17	B	–	–	–	–	
18	B	–	–	–	+	$<1 \times 10^2$
19	B	–	–	–	–	
20	B	–	–	–	–	
21	B	–	+	+	+	$<1 \times 10^2$
22	B	–	–	–	+	$<1 \times 10^2$
23	B	–	–	–	+	$<1 \times 10^2$
24	B	–	–	–	–	

–, negative; +, positive; group A, ankylos; group B, leone.





**Fig. 4.** Positive assemblies count at visual turbidity evaluation and at real-time PCR for group A and group B.

## DISCUSSION

Bacterial leakage from 2 prosthetic component surfaces and the respective proliferation was evaluated in the present study at 24, 48, and 72 hours. Such a short follow-up period was chosen according to data reported by da Silva-Neto et al<sup>37</sup> in a review of the methodology influence on microleakage studies. They advocated that monitoring periods over 7 days should be avoided because of the high risk of false negative observations. It was stated that any bacterial microleakage usually occurs within the first 3 days of evaluation. This may be a result of the reduction of the liquid and nutrients that are necessary for bacterial survival.

From the present study outcomes, a complete seal against microleakage can be provided by neither of the conometric connection systems when an insertion force of 50 N was applied. Furthermore, the bacterial sealing competence in terms of quantitative analysis did not differ between the 2 tested systems. Nevertheless, the microbial count was limited in both groups, hence indicating a narrow fluid passage through the interfaces.

In addition, despite the high number of *in vitro* studies investigating the implant to abutment interface,<sup>22,23,27,29</sup> the microleakage at the abutment-prosthesis is less investigated for cemented or screw-retained restorations.<sup>38</sup> Larger gaps can be expected at this level, as the prosthetic component is usually not prefabricated and thus less precise.<sup>38</sup> An *in vivo* study by Cosyn et al<sup>38</sup> aimed to evaluate the microbial contamination into intracoronary compartment and into abutment-implant connection. Cast-to-screw-retained fixed prostheses were

attached to the implant through transmucosal abutments. Heavily contaminated intracoronary compartments were observed. The abutment to prosthesis interface was stated as the principal pathway for bacterial leakage, through the restorative margin.

However, an improved performance in terms of microbial leakage amount can be obtained by the use of prefabricated components.<sup>39</sup> In addition, significantly improved marginal fit of implant-supported restorations was found when compared with conventional cast structures. Accordingly, in the present study, a low microbial count was obtained. Thus, the use of industrially manufactured components for the prosthetic connection is encouraged by the authors.

The sealing performance of Morse taper conometric systems as well as their retentive capability may be achieved by the wedge effect.<sup>40</sup> When a proper insertion force is applied, the coping cervical margin is slightly deformed; so that, elastic stress within both coping and abutment is generated.

Nevertheless, results of the present study cannot be compared with any data available in the literature, since the microleakage at conometric abutment to prosthesis interface was never previously tested.

However, the sealing capability of Morse taper connection was investigated at the implant-abutment interface in several studies. Microleakage of human saliva was compared with external-hexagon and internal-hexagon by do Nascimento et al.<sup>30</sup> In such study, microorganisms were found in the internal surfaces of all types of connections after 7 days of incubation. However, the lowest count of microorganisms was measured of Morse taper connection. Microbial passage through locking taper implant-abutment connection was evaluated in another study from Dibart et al<sup>23</sup>; 25 implant-abutment systems were used and divided into 2 phases of the experiment. Sealing capability against infiltration from the outer environment to the inner chamber was tested in the first phase; from the inner chamber to seeping out in the second. After incubation periods of 24 and 72 hours, no bacterial contamination for both phases was

observed; thus, the hermetic seal provided by locking taper connection was demonstrated.

Promising results were reported by clinical trials evaluating the performance of the Morse taper conometric interface supporting definitive restorations. In a 2-year study, on a total of 100 implants, no implant failed nor any significant change in marginal bone level was recorded.<sup>35</sup> Furthermore, optimal hygiene status was observed, as periimplant soft tissue inflammation was recorded in only 2 cases. Furthermore, in a recent 3-year report, the performance of 65 fixed partial prostheses supported by conometric connection abutments was evaluated: No disconnection of any lithium disilicate was assessed.<sup>41</sup>

From the present study outcomes, it can be hypothesized that a minimized bacterial leakage at the conometric prosthetic connection may reduce the periimplant tissues inflammation. Such speculation may be confirmed by medium- to long-term clinical trials on implant success. In addition, the absence of control groups in such *in vitro* study could represent a limitation. The comparison between bacterial leakage at conometric connection system and other types of prosthetic restorations, such as screwed or cemented, might be of interest.

## CONCLUSION

Within the limits of the present study, it can be concluded that a minimized bacterial infiltration may be allowed by both conometric interfaces for prosthetic connection. Microbial sealing performance did not differ between the 2 tested systems. The use of industrially manufactured components may explain the reduced microbial passage. The reduced bacterial passage at such interfaces may help maintain healthy periimplant tissues status.

Further research studies with larger samples are needed to find any difference between the test groups.

## DISCLOSURE

No author from the present study is involved, or has been involved, financially, directly or indirectly, in any of the products mentioned in this article.

## ROLES/CONTRIBUTIONS

## BY AUTHORS

All authors agree to be accountable for all aspects of work ensuring integrity and accuracy. E. Bressan contributed to conception, data interpretation, and drafted the manuscript. M. Stocchero contributed to data analysis and interpretation and drafted the manuscript. R. Jimbo contributed to data interpretation and critically revised the manuscript. C. Rosati contributed to data acquisition and analysis and critically revised the manuscript. E. Fanti contributed to data acquisition and analysis and drafted the manuscript. C. Tomasi contributed to conception, data analysis and interpretation, and revised the manuscript. D. Lops contributed to conception, data interpretation, and drafted the manuscript.

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