Microbial Leakage at the Implant-Abutment Connection Due to Implant Insertion Maneuvers: Cross-Sectional Study 5 Years Postloading in Healthy Patients

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The aim of this study was to test if stress on the prosthetic connection during insertion maneuvers can induce micro-warping at the implant connection. From September 2011 to July 2013, patients with implants loaded for at least 5 years that were placed with 2 different insertion implant mounters—MP (conventional) and ME (mountless)—were selected from all of those who had received dental implant therapy in the past and were attending routine check-up or spontaneous visits during the study period. Samples were obtained from inside the connection and the abutment surface using absorbent sterile paper tips. Quantitative real-time polymerase chain reaction was performed for total bacterial counts and loads of *Aggregatibacter actinomycetemcomitans* (*Aa*), *Porphyromonas gingivalis (Pg)*, *Tannerella forsythensis* (*Tf*), *Treponema denticola* (*Td*), *Prevotella intermedia* (*Pi*), *Peptostreptococcus micros* (*Pm*), *Fusobacterium nucleatum* (*Fn*), *Campylobacter rectus* (*Cr*), *Eikenella corrodens* (*Ec*), and *Candida albicans* (*Ca*). The analysis of variance test was used to test for differences. Nine patients (20 implants) were included in the MP group and 5 patients (10 implants) in the ME group. Regarding the red complex, *Tf* was seen in 80% and 30% of MP and ME implants, respectively ($P < .001$). Significant differences were also found in microbial load. For *Td*, proportions were 45% vs 10% ($P = .022$), with no significant differences at load levels. Regarding the orange complex, higher prevalence values were found in MP implants, although differences were nonsignificant. Microbial load levels for orange complex bacteria were higher for MP than ME, and these differences were statistically significant for *Fn* (4.94 vs 3.09; $P = .001$). Finally, *Ec* was detected only in the MP group, and *Ca* and *Aa* were not found in either group. Within its limitation (small sample size, retrospective analysis, indirect measurement method), the present study suggests that a mounter not affecting the prosthetic connection should be used to reduce microbial contamination of implants.

Key Words: titanium abutment, implant connection deformation, biomechanics, implant-prosthetic prognosis

INTRODUCTION

One factor that could jeopardize implant-prosthetic prognosis, especially for single-tooth restorations, is the lack of integrity of the implant-abutment junction (IAJ).[1] In fact, mechanical instability of the joint could be associated with biological complications.[2] Great efforts have been made in implant-connection improvement with regard to precision and stability[3,4]; however, tolerances are inherent to manufacturing processes, possibly leading to a contamination of the implant-abutment junction.[5]

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At the same time, although controversial, experimental and clinical studies quoted the importance of a high final insertion torque to favor implant osseointegration.[6,7] In fact, it appears to allow for mechanical implant adaption to the host bone until secondary stability is achieved. On the other hand, impaired primary implant stability has been shown to jeopardize the osseointegration process.

For a long period, implants were transported and inserted in the implant site osteotomy using mounters directly connected to the implant-abutment connection. From a theoretical point of view, high torque insertion values could be transferred to the prosthetic connection, causing its deformation. In fact, all commercial titanium alloys present relatively poor wear resistance. In particular, titanium surfaces in contact with each other or with other metals become distorted under conditions of sliding contact or friction.[8] These microdeformations could decrease connection stability and therefore increase microbiological contamination at the IAJ.[9] It could subsequently contaminate a fixture's surroundings and interfere with the health of peri-implant tissues.[10–12] The

Table 1

Subject and study site inclusion and exclusion criteria*

Subject inclusion criteria
Healthy peri-implant tissues: absence of bleeding on gentle probing (<0.25 N), PPD ≤ 5 mm, and absence of radiographic bone loss assessed in paralleled periapical radiographs (Lang & Berglundh 2011)
Uneventful functional loading for at least 5 years; the bridge must have not been removed during this time
Age > 18 y
Specific subject and site exclusion criteria
Presence of active periodontal or peri-implant pathology in any site of the mouth (diagnostic criteria: bleeding on gentle probing [<0.25 N] and PPD >3 mm in teeth and >5 mm in implants)
Use of antimicrobials during the 6 mo prior to the study
Pregnant and lactating patients
Patients refusing to sign an informed consent document or to participate in the study

*PPD indicates probing pocket depth.

presence of an increased bacterial reservoir in close relation to bone may have a role in the development of peri-implant tissue inflammation and bone loss.[13–15]

To theoretically prevent deformations, different insertion tools were developed to transfer torque stress settled during implant insertion to a nonrelevant prosthetic zone of the implant connection.

The present preliminary study aimed to test if stress on the implant connection during insertion maneuvers could induce micro-warping at the implant connection.

For this reason, the microbiota present inside the implant connection and in the peri-implant sulcus fluid of clinically healthy implants inserted with or without an insertion tool impacting the implant/abutment connection area was analyzed after at least 5 years of functional loading.

Materials and Methods

A cross-sectional study was performed in patients previously treated with dental implants, following the principles outlined in the Declaration of Helsinki. Patients were recruited between September 2011 and July 2013 at 2 private specialist centers (Rome and Viareggio, Italy). Patients with implants loaded at least 5 years that were placed with 2 different insertion implant mounters—MP (conventional) and ME (mountless)—were selected from all those who had received dental implant therapy at the mentioned departments in the past and who were coming to routine check-up or spontaneous visits during the study period. After being informed about the rationale of the study, patients signed a consent form. Inclusion and exclusion criteria are summarized in Table 1. All patients had participated in maintenance programs with routine control visits including oral professional prophylaxis every 6 to 12 months since their implants had been placed.

The specific inclusion criteria included patients with implants presenting the same type of implant-abutment connections (internal hexagon with external collar, Premium-Kohno, Sweden&Martina, Padua, Italy) with 5 years of functional loading and patients with implants inserted using a mounter directly connecting with prosthetic connection (MP) and patients with implants inserted using a mounter tool not impacting the prosthetic area (ME; Figure 1).

Microbiological sampling

Sampling for microbiological analysis from all groups was performed by a single researcher.

Sampling was performed using GUIDOR Perio-Implant Diagnostic Test kits (Sunstar Iberia S.L.U, Barcelona, Spain), consisting of 5 sterile absorbent paper tips and a 2-mL sterile empty Eppendorf tube. The supragingival plaque was eliminated from implants and teeth using a curette or cotton roll, without penetrating the gingival or peri-implant sulcus. Cotton rolls were used for relative isolation. To collect samples of the implant connection, prostheses and abutments were carefully removed, while trying to avoid contamination. One drop of RNA- and DNA-free water (Water Molecular Biology Reagent, code W4502, Sigma, St Louis, Mo) was placed inside the implant connection, and 3 paper tips were inserted for 30 seconds. The connection surface of the abutment was wetted with a drop of RNA- and DNA-free water and smeared with 2 paper tips. Subsequently, the paper tips were placed into the Eppendorf tubes and sent for microbiological analysis to the laboratory (Institut Clinident SAS, Aix en Provence, France) using the provided mailing envelopes.

Quantitative real-time polymerase chain reaction assays

Quantitative real-time polymerase chain reaction (qRT-PCR) was carried out for total bacterial counts of 10 pathogens: *Aggregatibacter actinomycetemcomitans* (*Aa*), *Porphyromonas gingivalis* (*Pg*), *Tannerella forsythensis* (*Tf*), *Treponema denticola* (*Td*), *Prevotella intermedia* (*Pi*), *Peptostreptococcus micros* (*Pm*), *Fusobacterium nucleatum* (*Fn*), *Campylobacter rectus* (*Cr*), *Eikenella corrodens* (*Ec*), and *Candida albicans* (*Ca*). The qRT-PCR assays were performed in a volume of 10 μL composed of 1× QuantiFast SYBR Green PCR (Qiagen, Germany), 2 μL of DNA extract, and 1 μM of each primer. The species-specific PCR primers used in this study were provided by Institut Clinident SAS and manufactured by Metabion GmbH (Martinsried, Germany).

Assays were carried out on the Rotor-Gene Q thermal cycling system (Qiagen) with the following program: 95°C for 5 minutes, followed by 40 cycles of 10 seconds at 95°C, 10 seconds at 60°C, and 35 seconds at 72°C. A final melt curve analysis (70°C to 95°C in 1°C steps for 5-second increments) was done. Fluorescence signals were measured every cycle at the end of the extension step and continuously during the melt curve analysis. The resulting data were analyzed using Rotor-Gene Q Series software (Qiagen). Serial dilutions of standard DNA provided by Institut Clinident SAS were used in each reaction as external standards for absolute quantification of the targeted pathogens.

Statistical analysis

The mean prevalence of bacterial counts was obtained for each group. Total bacterial loads were transformed (log) before computations. Statistical analysis was performed with analysis

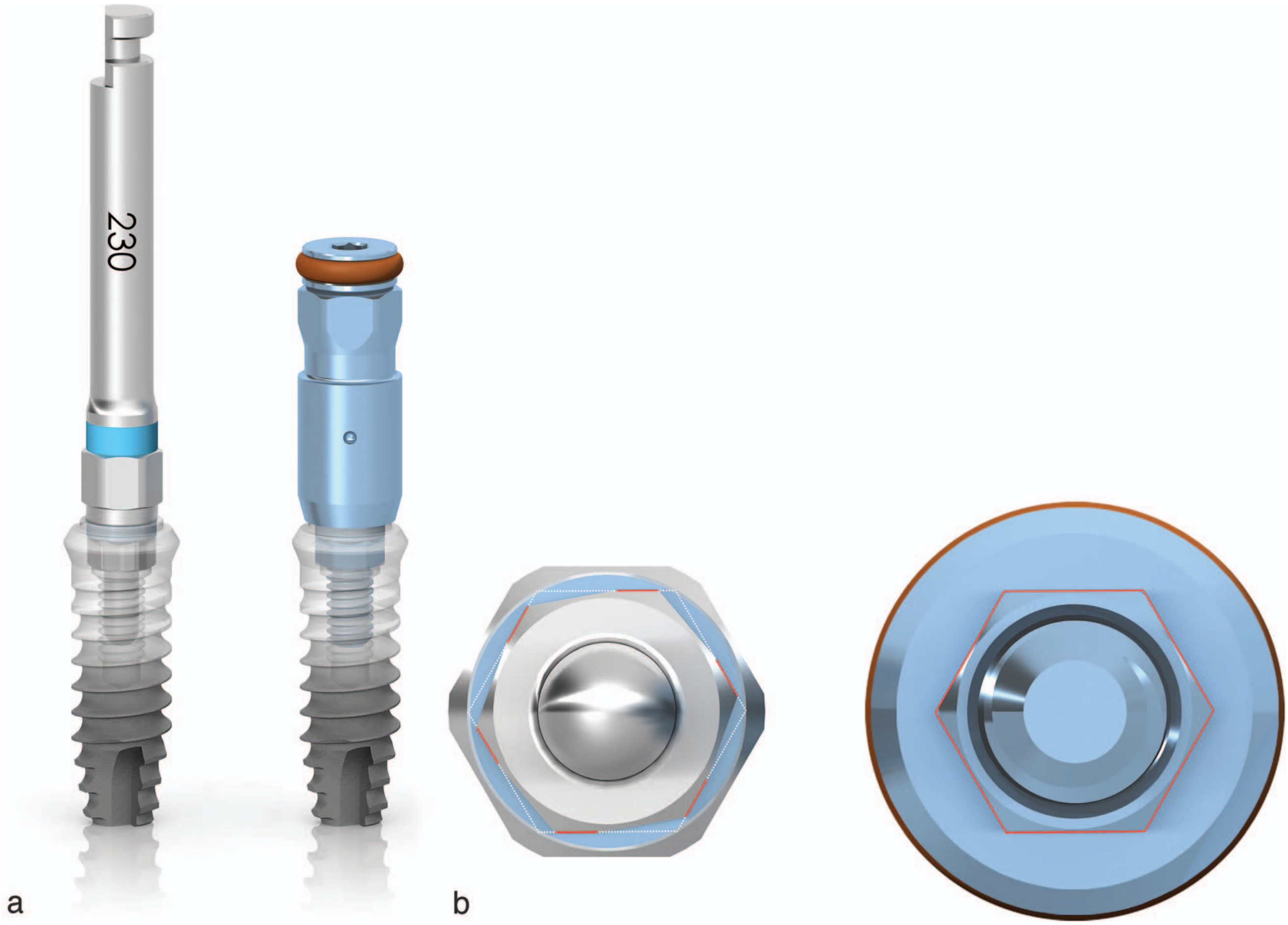

Figure 1. (a) Schematic drawing of ME and MP insertion modes. (b) Contact area between the mounters (ME and MP) and the implant connection.

of variance–type test using the Brunner–Langer model because of inequalities in patient and implant sizes between the MP and ME groups, assuming group as a between-subject factor and implant as a within-subject factor.

The connection type and contamination at patient level were considered. All tests were performed at a 5% level of significance.

Results

Description of the study sample

A total of 29 patients previously treated with 59 dental implants were checked during the study period. Nine patients were excluded: 7 had taken systemic antibiotics during the 3 months prior to the microbiological sampling, and 2 patients refused to participate.

The final sample consisted of 20 patients and 43 implants divided in 2 groups: 10 patients and 20 implants in the MP group and 10 patients and 23 implants in the ME group. Data are presented in Table 2.

The microbial prevalence for each species can be seen in Figure 2. Mean total bacterial loads ($\log_{10}$) are presented in Figure 3. Regarding the red complex, *Tf* was seen at 80% of MP implants compared with 30% of ME implants ($P < .001$). Significant differences were also observed for bacterial loads of this species (3.78 vs 1.23; $P < .001$). For *Td*, proportions were 45% vs 10% ($P = .022$), but differences in load levels were nonsignificant ($P = .065$). Forty percent of MP implants presented positively for all 3 red complex bacteria simultaneously, in contrast with no implant showing this in the ME group ($P = .001$). Regarding the orange complex, higher prevalence values were found in MP implants, although differences were nonsignificant. Microbial load levels for orange complex bacteria were again higher for MP than ME, with these differences being statistically significant for *Fn* (4.94 vs 3.09; $P = .001$). Finally, the pathogen *Ec* was seen only in the MP group, and *Ca* and *Aa* species were not found in either group.

Discussion

The present work reveals that the internal connection of titanium implants could be subjected to deformation after implant insertion procedures, which involves a potential instability of the implant-abutment complex. These deforma-

TABLE 2

Descriptive summary for bacteria prevalence and load (log) by type of connection: results for Brunner-Langer analysis of variance–type test (*P* value)

	Prevalence (%) and Log of Load (Mean ± SD)		
	MP	ME	*P* Value
Aa	0	0	1.000
	—	—	
Pg	40	30	.567
	2.00 ± 2.57	1.03 ± 1.69	.201
Tf	80	30	<.001***
	3.78 ± 2.05	1.23 ± 2.03	<.001***
Td	45	10	.022*
	2.20 ± 2.51	0.55 ± 1.72	.065
Pg+Tf	40	20	.252
Pg+Td	40	0	.001**
Tf+Td	45	10	.022*
Pg+Tf+Td (red c.)	40	0	.001**
Pi	25	20	.763
	1.46 ± 2.62	0.94 ± 1.99	.579
Pm	85	70	.483
	4.05 ± 2.12	2.76 ± 2.17	.131
Fn	95	80	.256
	4.94 ± 1.55	3.09 ± 1.81	.001**
Pi+Pm	25	20	.763
Pi+Fn	25	20	.763
Pm+Fn	85	60	.216
Pi+Pm+Fn (orange c.)	25	20	.763
Cr	55	20	.058
	2.88 ± 2.70	1.01 ± 2.15	.043*
Ec	25	0	.025*
	1.13 ± 2.03	—	.033*
Ca	0	0	1.000
	—	—	
Total	100	100	1.000
	7.42 ± 1.10	5.95 ± 0.84	<.001***

$^*P < .05$; $^{**}P < .01$; $^{***}P < .001$.

tions appear to be microbiologically relevant after 5 years of loading.

Microbial penetration through the IAJ and colonization of the connection's inner portion are clearly demonstrated by in vitro[3,4] and in vivo studies.[16] A bacterial reservoir may establish inside the implant that, in the long term, could seriously affect the health of peri-implant tissue.[11] The occurrence of bacterial leakage at the internal surface of implants through the IAJ is, in fact, one of the parameters for analyzing the degree of quality in the fabrication of these connections.[17] However, deformations of the implant connection due to the fixture insertion could enhance the instability of the implant-prosthetic ensemble, leading to a high risk of clinical complications.

According to the present data, for a clinical point of view, it seems wise to diminish as much as possible the insertion torque or use a mounter that does not impact the prosthetic connection.

This study, which was the first aimed to analyze the clinical effect of the stress at the implant-abutment interface during insertion procedures, is in agreement with other in vitro studies present in the literature. In fact, as demonstrated by Imam et al,[8] excessive rotation strength could lead to implant-abutment interface failure. At the same time, in another in vitro study, Kwon et al[9] demonstrated that even under 45-Ncm insertion torque, the rotational freedom between an implant and its abutment was significantly increased.

However, the limits of the study were the retrospective recruitment method, the small sample size, the indirect measurement method, and the limited amount of bacterial species studied. Furthermore, since only 1 implant brand was tested, performing the same tests on a bigger sample size and different implant/connection design and materials would help to generalize the presented results. In fact, both implants and components used in the present study were of grade 4 cp. titanium. It could be hypothesized that the use of grade 5 titanium or zirconia implants, especially in conjunction with mounters of cp. titanium, could eliminate deformations.

CONCLUSIONS

In this preliminary study, differences were found in microbiological contamination of the implant-abutment connection between implants inserted using a mounter directly fitting the prosthetic area of the connection and a mounter not impacting the prosthetic area. These results should encourage clinicians to minimize as much as possible insertion torque or to use mounters not impacting the prosthetic connection.

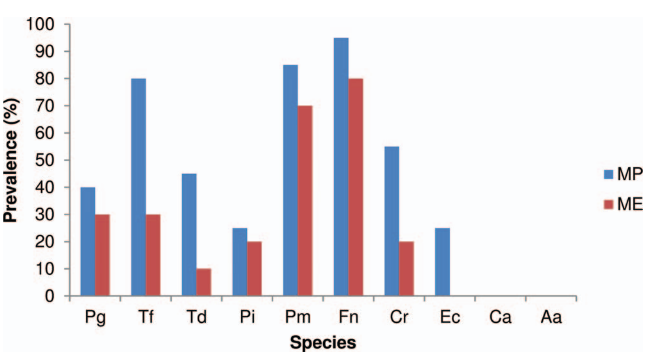

FIGURE 2. Microbial prevalence (%) at MP and ME groups for each species.

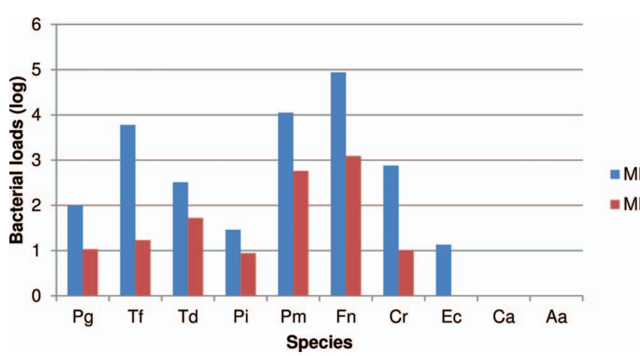

FIGURE 3. Bacterial load ($\log_{10}$) at MP and ME groups for each species.

Abbreviations

Aa: *Aggregatibacter actinomycetemcomitans*
Ca: *Candida albicans*
Cr: *Campylobacter rectus*
Ec: *Eikenella corrodens*
Fn: *Fusobacterium nucleatum*
IAJ: implant-abutment junction
Pg: *Porphyromonas gingivalis*
Pi: *Prevotella intermedia*
Pm: *Peptostreptococcus micros*
qRT-PCR: quantitative real-time polymerase chain reaction
Td: *Treponema denticola*
Tf: *Tannerella forsythensis*

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