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Salivary bacterial leakage into implant-abutment connections: preliminary results of an *in vitro* study

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Abstract. – OBJECTIVE: The occurrence of bacterial leakage in the internal surface of implants, through implant-abutment interface (IAI), is one of the parameters for analyzing the fabrication quality of the connections. The aim of this in vitro study is to evaluate two different types of implant-abutment connections: the screwed connection (Group 1) and the cemented connection (Group 2), analyzing the permeability of the IAI to bacterial colonization, using human saliva as culture medium.

PATIENTS AND METHODS: A total of twelve implants were tested, six in each experimental group. Five healthy patients were enrolled in this study. Two milliliters of non-stimulated saliva were collected from each subject and mixed in a test tube. After 14 days of incubation of the bacteria sample in the implant fixtures, a PCR-Real Time analysis was performed. Fisher's exact test was used to compare the proportions of implant-abutment assembled structures detected with bacterial leakage. Differences in the bacterial counts of the two groups were compared using the Mann-Whitney U test. A p value < 0.05 was considered significant.

RESULTS: The results showed a decreased stability with the screwed implant-abutment connections compared to the cemented implant-abutment connections. A mean total bacterial count of 1.2E+07 (± 0.25E+07) for Group 1 and of 7.2E+04 (± 14.4E+04) for Group 2 was found, with a high level of significance, $p = .0001$.

CONCLUSIONS: Within the limitations of this study it can be concluded that bacterial species from human saliva may penetrate along the implant-abutment interface in both connections, however the cemented connection implants showed the lowest amount of bacterial colonization.

Key Words:

Implant-abutment interface, Screwed connection, Cemented connection, Peri-implant, Bacterial adhesion.

Introduction

Dental implant restoration has been widely accepted as one of the treatment modalities to replace missing teeth and to restore human masticatory functions[1].

Elevated success rates have been described in the current literature for long-term treatment with osseointegrated dental implants[2-4]. Usually, these high success rates have been related to immediate stabilization processes, mainly associated to the quality and characteristics of the implants used[5]. Implant primary stability has been reported as the key factor to improve the implant survival rate.

In spite of the excellent success rates in osseointegrated implant rehabilitations, failures have been described in literature. The implant failures are essentially related to mechanical and microbiological factors that frequently act in association[6,7]. The unfavourable occlusion and diversity of microorganisms inhabiting the oral cavity, especially those related to periodontal diseases, are the main factors associated to late implant complications[8,9].

The implant-abutment connection represents the weakest point of the dental endosseous implant, as it must resist maximal and permanent chewing forces as well as bacterial infiltration.

Two-piece implants unavoidably present a micro-gap between the implant and the abutment. When a prosthetic abutment is connected to a fixture, a micro-gap is created between the components, due to an inadequate fit between implant and abutment. Microorganisms may grow into this implant-abutment interface (IAI) micro-gap and establish a bacterial reservoir resulting in an area of inflamed soft tissue, which faces the fixture-abutment junction[10-12].

This inadequate fit between the implant and the abutment may be considered a risk factor

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similar to that in poorly adapted dental restorations, capable of leading to clinical and microbiological alterations in the peri-implant tissues.

Callan et al[13], analyzing the size of the fixture abutment interface, found mean values between 30 µm and 135 µm; Dellow et al[14] between 0 and 7.15 µm; and Jansen[15] between 1 and 10 µm. Due to the existence of the interface, the possibility of fluid and microorganism exchanges is probable.

The implant abutment interface can allow the passage of fluids and bacteria, irrespective of the implant system (with tapered or flat connections). Well fitting interfaces too (smaller than 5 µm) were incapable of preventing bacterial leakage and colonization of the internal implant surface. A large variety of microorganisms appear to have the ability to penetrate at the fixture abutment interface and reach the inside of implants, ranging from Gram-positive coccus to Gram-negative rods. *Streptoccocus sanguis* presents a mean size ranging between 0.8 µm and 1 µm and *Escherichia coli* presents a mean size ranging between 1.1 µm and 1.5 µm in diameter and 2 µm and 6 µm in length, being considered of medium size in comparison with the oral micro-flora. These characteristics enable bacterial leakage at interfaces with maladjustment within the values described in the literature[15].

The potential colonization of the internal connection through the implant-abutment micro-gap is probably related to multi-factorial conditions, i.e., the precision fit between the implant components, which is associated with the implant system design; the torque used to connect the components; the repeated screw loosening and retightening; and the loading force when the implant is in function[16-22].

Several studies have demonstrated the presence of a micro-gap between implant and abutment and the occurrence of bacterial leakage in IAI. However, artifices can be created to render the clinical significance of this gap negligible. The supra-crestal position of the interface, the adaptation torque of the screw to the implant, use of silicone to seal the interface, and the system design are the main findings that are clinically significant. The occurrence of bacterial leakage in the internal surface of implants, through IAI, is one of the parameters for analyzing the fabrication quality of the connections.

In this contest, the connection design and the type of system used to connect the implant with the abutment, are both very important factors.

The purpose of this *in vitro* study is to compare different implant-abutment connection based on their permeability to the bacterial colonization, evaluating the detection frequency of bacterial leakage from human saliva through the implant-abutment interface.

The investigators hypothesize that screwed implant-abutment connection show a higher colonization by bacteria than the cemented connection.

Patients and Methods

In this study, a total of twelve implants were tested, six in each experimental group. Group 1 consisted of fixtures (Winsix®, BioSAF IN, Ancona, Italy), 4.5 mm in diameter, with an internal hexagon connected to the abutment with a retained screw; and Group 2 consisted of fixtures (Bone System®, Milan, Italy), 4.1 mm in diameter, with an internal hexagon connected to the abutment with a sealant (Panavia 21, J. Morita USA Inc, Tustin, CA, USA).

Under aseptic conditions, in a laminar flux hood and using sterile instruments and gloves, the abutments, previously autoclaved at 121°C for 30 minutes, were attached to sterile implants following the manufacturer's instructions.

For Group 1 the abutments were screwed to fixtures using a dynamometric ratchet with a torque of 25 Ncm.

For Group 2 the abutments were cemented into the fixtures through a specific collar inserted into the implant, using a device that ensures the inserting pressure. In Group 2 the cement was mixed, at room temperature, according to the manufacturer's protocol and applied on the axial surface of the internal portion of the implants to minimize the hydrostatic pressure during its hardening. Abutments were cemented onto the implants with a load of 5 kg maintained for 10 minutes.

All the procedures were carried out by the same expert investigator.

Saliva Collection

The study was open to all subjects who met specific inclusion and exclusion criteria (Table I) and provided signed informed consent according to the World Medical Association's Declaration of Helsinki.

Five healthy subjects, aged between 31 and 60 years (mean age 37 ± 9.09 years), were enrolled in this study. Two milliliters of non-stimulated saliva were collected from each subject and

Table I. Inclusion criteria.

No clinical signs of oral mucosa diseases
Gingival sulci less than 3 mm deep
No clinical signs of inflammation
No caries or active white spot lesions
No pregnant or lactating
No periodontal antibiotic treatment during the previous 3 months
No smokers
No systemic diseases
Written consent

mixed in a test tube.

In addition, samples of supra-gingival biofilm from the subjects' first maxillary and mandibular molars were taken with individual curettes and added to the test tube.

The selected subjects had no clinical sign of disease in their oral mucosa. The gingival sulci were less than 3 mm deep and showed no clinical signs of inflammation. There were no caries or active white spot lesions on the teeth. Patients who were currently pregnant or lactating, had received periodontal antibiotic treatment during the previous 3 months, were current smokers, or had any systemic disease that could influence the periodontal status were excluded from study (Table I).

The collected saliva samples were taken every morning for 14 days, making sure that the ordinary oral hygiene remained unvaried.

Microbiological Assessment

Before the implant-abutment connection, samples from the internal parts of the implants were collected with sterile paper points to be used as negative controls for the bacterial contamination.

Under aseptic conditions, in a laminar flux hood and using sterile instruments and gloves, the abutments were attached to implants following the manufacturer's instructions.

Next, the implants and the abutments were washed twice with a sterile physiological solution (0.9% NaCl) and dried with sterile gauze pads. Five assembled structures for each group were immersed into micro-tubes containing 200 µl of human saliva. This supernatant volume was sufficient enough so to ensure that the micro-gap between the implant and abutment (IAI) was totally covered by saliva.

One assembled structure per group was immersed into micro-tubes containing 200 µl of sterile physiological solution (0.9% NaCl) and was used as negative control.

The micro-tubes were incubated at 37°C in anaerobic conditions for 14 days.

Every morning the plaque and saliva within the micro-tubes were partially changed with new saliva samples.

After incubation, the assembled structures were aseptically disconnected, placed on sterile absorbing paper, washed with NaCl 0.9%, and externally dried with sterile gauzes.

The samples from the internal parts of the implants were collected using a kit, consisting of 10 sterile absorbent paper tips and sterile Eppendorf tubes (Eppendorf Tubes®, Hamburg, Germany).

One drop of RNA-and DNA-free water (Water Molecular Biology Reagent code W4502, Sigma-Aldrich, Saint Louis, MI, USA) was placed inside the implant connection and three paper tips were insert for 30s.

The connection surface of the abutment was wet with a drop of RNA-and DNA-free water and smeared with two paper tips.

The papers tips were placed into the Eppendorf tubes and were sent for microbiological analysis to the laboratory Institut Clinident SAS (Aix en Provence, France) inside provided mailing envelopes.

Quantitative Real-Time PCR Assays

Quantitative real-time PCR was carried out for the Total Bacterial Count (TBC) and for 10 pathogens: *Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tennerella forsythensis (Tf), Treponema denticola (Td), Prevotella intermedia (Pi), Peptostreptococcus micros (Pm), Fusobacterium nucleatum (Fn), Campylobacter rectus (Cr)* and *Eikella corrodens (Ec);* and *Candida Albicans (Ca).*

Quantitative real-time PCR assays were performed in a volume of 10 1l composed of 1 9 QuantiFast® SYBR® Green PCR (Qiagen, Hilden, Düsseldorf, Germany), 2 1l of DNA extract and 1 1M of each primer. The species-specific PCR primers used in this study were provided by Institut Clinident SAS (Aix en Provence, France) and manufactured by Metabion GmbH (Metabion international AG, Planneg, München, Germany). The bacterial primers used derived from previously published ribosomal 16S sequences and have been adapted to the real-time PCR conditions.

Assays were carried out on the RotorGene® Q thermal cycling system (Qiagen, Hilden, Düsseldorf, Germany) with the following program: 95°C for 5 min, followed by 40 cycles of 10 s at 95°C, 10 s at 60°C, and 35 s at 72°C. A final

melt curve analysis (70 to 95°C in 1°C steps for 5s increments) was performed. Fluorescence signals were measured at the end of the extension step of every cycle and continuously during the melt curve analysis. The resulting data were analyzed using Rotor-Gene® Q Series software (Qiagen, Hilden, Düsseldorf, Germany).

Serial dilutions of bacterial standard DNA provided by Institut Clinident SAS (Aix en Provence, France) were used in each reaction as external standards for absolute quantitation of the targeted bacterial pathogens. Standard bacterial strains used for standard DNA production were obtained from DSMZ (Braunschweig, Germany), CIP Collection of Institut Pasteur (Paris, France) or from BCMM/LMG Bacteria Collection (Ghent, Belgium): *Aa* (DSM No. 8324), *Pg* (DSM No. 20709), *Tf* (CIP No. 105220), *Td* (DSM No. 14222), *Pi* (DSM No. 20706), *Pm* (DSM No. 20468), *Fn* (DSM No. 20482), *Cr* (LMG No. 18530), *Ec* (DSM No. 8340).

Statistical Analysis

Descriptive analysis was used to summarize the data, mean values and standard deviations (SD) were calculated. In addition, the total number of implants per group exhibiting bacterial colonization of the IAI microgap was calculated. Fisher's Exact test was used to compare the proportions of implant-abutment assembled structures detected with bacterial leakage. Differences in the bacterial counts of the two groups were compared using the Mann-Whitney *U* test.

A p value < 0.05 was considered significant. A specific statistical software (IBM SPSS V10 Statistics, IBM, Armonk, USA) was used for data analysis.

Results

In this study a total of 12 implants were tested, 6 for each group. Implants were assembled and inserted in tubes containing human saliva and were incubated at 37°C for 14 days. After 14 days, with partially changed saliva samples every morning, implant-abutments were disassembled and the bacteria samples were performed using sterile paper points inserted into the implant for 30 seconds. The samples were sent to Institut Clinident SAS (Aix en Provence) and analyzed by real-time PCR.

PCR methodology provided Genomic DNA extraction from the samples and tested for microorganisms associated with peri-implantitis. The DNA was tested by Polymerase Chain Reaction (PCR) amplification followed by fluorescence detection and quantitation.

The results show a low permeability to bacteria for Group 2 implants, with cemented implant-abutment connections, and a high permeability to bacteria for the Group 1, with screwed implant-abutment connection, as showed in Figure 1 and Figure 2.

After 14 days, bacterial contamination was observed in a total of 6 assembled structures out of the 10, 5 were in Group 1, with screwed connections, and only 1 assembled structure was of Group 2, with the cemented connection. The contamination, was therefore of 100% for the Group 1 implants and of 20% for Group 2, but was not statistically significant; p value = .074.

No contamination by bacteria was observed for implants immersed in sterile physiological solution for either groups.

The total bacterial count was 1.2E+07 (± 0.25E+07) for Group 1 versus 7.2E+04 (±14.4E+04) for Group 2, with a high level of significance, p = .0001.

For Group 1, the bacterial contamination was positive for all tested bacteria except *Aggregatibacter actinomycetemcomitans* and *Candida albicans,* but all bacteria were below the pathogenicity threshold (Figure 3).

For Group 2, the bacterial contamination was positive for only two bacteria *Porphyromonas gingivalis* and *Fusobacterium nucleatum,* and well below the pathogenicity threshold (Figure 4).

Discussion

The precise mechanism responsible for crestal bone remodeling in 2-piece implants is not known. One of the factors causing most concern is the IAI (Implant-abutment interface). If the interface of the implant and abutment is not precise and does not fit adequately, it can cause the formation of a micro-gap. Microbial infiltration through the micro-gap, which inevitably exists between implants and abutments, and the colonization of the connection's inner portion are demonstrated by *in vitro* and *in vivo* studies.

A bacterial reservoir may establish inside the implant, which with time can seriously affect the health of peri-implant tissue.

According to Broggini et al[23], the precision of

Bacteria	Pathogenic load*	Pathogenic threshold**	Status***	% / Total Bacterial Count****
Aggregatibacter actinomycetemcomitans	0,0E+00	1,0E+03	-	0,00
Porphyromonas gingivalis	3,9E+04	1,0E+05	+	0,33
Tannerella forsythia	7,3E+02	1,0E+05	+	0,006
Treponema denticola	6,7E+03	1,0E+05	+	0,06
Prevotella intermedia	7,1E+04	1,0E+05	+	0,60
Parvimonas micra	1,7E+05	1,0E+06	+	1,45
Fusobacterium nucleatum	1,4E+05	1,0E+07	+	1,22
Campylobacter rectus	2,6E+05	1,0E+06	+	2,16
Eikenella corrodens	6,7E+03	1,0E+07	+	0,06
Candida albicans	0,0E+00	N/A	**Negative**	N/A
Total Bacterial Count	1,2E+07			

Figure 1. Group 1, screwed implant-abutment connection, and Group 2, cemented implant-abutment connection, real-time PCR average results. *Pathogenic load: the amount of detected bacteria in the sample; **Pathogenic threshold: Represents a specific microbiological pathogenic load above which antibiotic therapy is recommended in order to reduce risk of tooth or implant attachment loss (periodontal disease or peri-implantitis). ***Status: levels of microbiological pathogenic load: -Absent; +Moderate and less than the pathogenic load threshold; ++High and more the pathogenic load threshold. Associated with aggressive forms of disease; +++Very high and more than 10 times above the pathogenic load threshold; ++++Very strong association with aggressive forms of disease and loss of bone attachment; Negative, absence of *Candida albicans*/Positive, presence of *Candida albicans*. ****%Total Bacterial Count: relative proportion of a specific bacteria versus total bacterial count; N/A not available.

Bacteria	Pathogenic load*	Pathogenic threshold**	Status***	% / Total Bacterial Count****
Aggregatibacter actinomycetemcomitans	0,0E+00	1,0E+03	-	0,00
Porphyromonas gingivalis	6,6E+02	1,0E+05	+	0,92
Tannerella forsythia	0,0E+00	1,0E+05	-	0,00
Treponema denticola	0,0E+00	1,0E+05	-	0,00
Prevotella intermedia	0,0E+00	1,0E+05	-	0,00
Parvimonas micra	0,0E+00	1,0E+06	-	0,00
Fusobacterium nucleatum	2,8E+02	1,0E+07	+	0,39
Campylobacter rectus	0,0E+00	1,0E+06	-	0,00
Eikenella corrodens	0,0E+00	1,0E+07	-	0,00
Candida albicans	0,0E+00	N/A	**Negative**	N/A
Total Bacterial Count	7,2E+04			

Figure 2. Group 1, screwed implant-abutment connection, and Group 2, cemented implant-abutment connection, real-time PCR average results. *Pathogenic load: the amount of detected bacteria in the sample; **Pathogenic threshold: Represents a specific microbiological pathogenic load above which antibiotic therapy is recommended in order to reduce risk of tooth or implant attachment loss (periodontal disease or peri-implantitis). ***Status: levels of microbiological pathogenic load: -Absent; +Moderate and less than the pathogenic load threshold; ++High and more the pathogenic load threshold. Associated with aggressive forms of disease; +++Very high and more than 10 times above the pathogenic load threshold; ++++Very strong association with aggressive forms of disease and loss of bone attachment; Negative, absence of *Candida albicans*/Positive, presence of *Candida albicans*. ****%Total Bacterial Count: relative proportion of a specific bacteria versus total bacterial count; N/A not available.

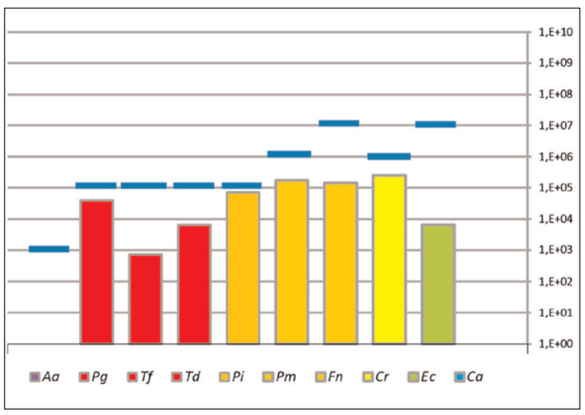

Figure 3. Group 1 and Group 2 bacterial leakage, average results. Aa: Aggregatibacter actinomycetencomitans; Pg: *Porphyromonas gingivalis*; Tf: *Tannerella forsythia*; Td: *Treponema denticola*; Pi: *Prevotella intermedia*; Pm: Parvimonas micra; Fn: *Fusobacterium nucleatum*; Cr: *Campylobacter rectus*; Ec: *Eikenella corrodens*; Ca: *Candida albicans*.

the space in the IAI at the level of the bone crest is associated with reduction in the accumulation of inflammatory peri-implant cells and minimum bone loss. Rangert et al[24], McCartney et al[25], and May et al[26] also stated that the accurate assemblies of implant components and the precision of fit of the prosthesis to the implant is absolutely essential for the long-term survival of dental implants and the preservation of the supporting bone.

In rehabilitations with implants, external prosthetic connections of the external hexagonal type, and internal connections, such as hexagonal, tapered (Morse Cone), or both in combination are used. Tapered connections appear to have a superior stability when compared with the external hexagonal type. According to Dibart et al[27], the frictional connection of a tapered pillar consists of a cold, metal-to-metal shoulder; which seals and thus makes the IAI very narrow so to try to prevent the passage of bacteria.

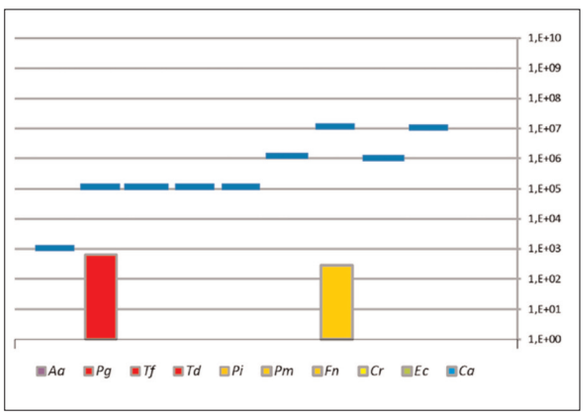

Figure 4. Group 1 and Group 2 bacterial leakage, average results. Aa: *Aggregatibacter actinomycetencomitans*; Pg: *Porphyromonas gingivalis*; Tf: *Tannerella forsythia*; Td: Treponema denticola; Pi: *Prevotella intermedia*; Pm: *Parvimonas micra*; Fn: *Fusobacterium nucleatum*; Cr: Campylobacter rectus; Ec: *Eikenella corrodens*; Ca: *Candida albicans.*

Prosthetic connections with a better sealing capacity of the IAI have been investigated in order to eliminate bacterial leakage. Cemented pillars, varnish containing 1% chlorhexidine, silicon sealant and the silicone ring have been assessed. The authors verified that the cement-retained implant-abutments offer better results relating to fluid and bacterial permeability compared with screw-retained implant-abutments. Besimo et al[28] observed no contamination until 11 weeks at the internal surface of implants when chlorhexidine varnish was applied at the IAI, in internal hexagon connection; however, Duarte et al[29] when assessing varnish containing 1% chlorhexidine and a silicone sealant, verified that these materials were incapable of preventing bacterial leakage.

According to Quirynen et al[7], the bone crest loss associated with dental implants is directly related to the existence of IAI at the alveolar crest, which could favor the maintenance of a chronic inflammatory process in the area, due to bacterial accumulation.

Marginal bone stability is an important factor for the longevity of implants. Horizontal and vertical bone loss is generally associated with the presence of a space at the IAI and peri-implant bacterial infection.

Therefore, it is very important to know the risk factors, as well as their etiologies in determining bone loss[30].

Hermann et al[31] concluded that radiographic and histologic analyses indicated that the alteration at the bone crest depends on the characteristics of the implant surface, presence, absence and location of a gap.

Tonetti and Schmid[32] conducted a literature review regarding pathogenic processes that lead to osseointegration failure. The late failures that occurred after established osseointegration were classified into disturbances of biomechanical equilibrium (overload) and alterations in the host-parasite equilibrium (infection). The stability of osseointegration depends on a dynamic equilibrium in biomechanical terms, and on inter-

actions between the host-parasite.

In this study two different implant-abutment connection types were compared, one with a screwed implant-abutment connection and the other with a cemented implant-abutment connection.

The results, in agreement with Schmitt et al[33], showed a decreased stability for the screwed implant-abutment connection compared to the cemented implant-abutment connection.

In this *in vitro* study, the screwed implant-abutment connection showed an higher colonization by bacteria than the cemented connection, with 100% implants colonized by bacteria versus 20%, and a higher average total count (1.2E+07 *versus* 7.2E+04). The difference was statistically significant with a p value < .05.

Conclusions

To the best of the author's knowledge, this study is one of the few that compares screwed connection and cemented connection, based on their permeability to the bacterial colonization, evaluating the detection frequency of bacterial leakage from human saliva through the implant-abutment interface.

Within the limitations of the relatively small sample of this work, it can be concluded that bacterial species from human saliva may infiltrate along the implant-abutment interface in both connections, however cemented connection implants showed the lowest amount of bacterial colonization.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Faggioni CM Jr, Giannakopoulos NN, Listl S. How strong is the evidence for the need to restore posterior bounded edentulous spaces in adults? Grading the quality of evidence and the strength of recommendations. J Dent 2011; 39: 108-116.
2) Lang NP, Pjetursson BE, Tan K, Brägger U, Egger M, Zwahlen M. A systematic review of the survival and complication rates of fixed partial dentures (FPDs) after an observation period of at least 5 years. II. Combined tooth-implant-supported FPDs. Clin Oral Implants Res 2004; 15: 643-653.
3) Pjetursson BE, Brägger U, Lang NP, Zwahlen M. Comparison of survival and complication rates of tooth-supported fixed dental prostheses (FDPs) and implant-supported FDPs and single crowns (SCs). Clin Oral Implants Res 2007; 18: 97-113.
4) Jung RE, Pjetursson BE, Glauser R, Zembic A, Zwahlen M, Lang NP. A systematic review of the 5-year survival and complication rates of implant-supported single crowns. Clin Oral Implants Res 2008; 19: 119-130.
5) Heberer S, Hildebrand D, Nelson K. Survival rate and potential influential factors for two transitional implant systems in edentulous patients: a prospective clinical study. J Oral Rehabil 2011; 38: 447-453.
6) Piattelli A, Scarano A, Paolantonio M, Assenza B, Leghissa GC, Di Bonaventura G, Catamo G, Piccolomini R. Fluids and microbial penetration in the internal part of cement-retained versus screw-retained implant-abutment connections. J Periodontol 2001; 72: 1146-1150.
7) Quirynen M, De Soete M, van Steenberghe D. Infectious risks for oral implants: a review of the literature. Clin Oral Implants Res 2002; 13: 1-19.
8) Brägger U, Karoussis I, Persson R, Pjetursson B, Salvi G, Lang N. Technical and biological complications/failures with single crowns and fixed partial dentures onimplants: a 10-year prospective cohort study. Clin Oral Implants Res 2005; 16: 326-334.
9) Covani U, Marconcini S, Crespi R, Barone A. Bacterial plaque colonization around dental implant surfaces. Implant Dent 2006; 15: 298-304.
10) Quirynen M, Vogels R, Peeters W, van Steenberghe D, Naert I, Haffajee A. Dynamics of initial subgingival colonization of 'pristine' peri-implant pockets. Clin Oral Implants Res 2006; 17: 25-37.
11) Callan DP, Cobb CM, Williams KB. DNA probe identification of bacteria colonizing internal surfaces of the implant-abutment interface: a preliminary study. J Periodontol 2005; 76: 115-120.
12) Ericsson I, Persson LG, Berglundh T, Marinello CP, Lindhe J, Klinge B. Different types of inflammatory reactions in peri-implant soft tissues. J Clin Periodontol 1995; 22: 255-261.
13) Callan DP, O'Mahony A, Cobb CM. Loss of crestal bone around dental implants: a retrospective study. Implant Dent 1998; 7: 258-266.
14) Dellow AG, Driessen CH, Nel HJ. Scanning electron microscopy evaluation of the interfacial fit of interchanged components of four dental implant systems. Int J Prosthodont 1997; 10: 216-221.
15) Jansen VK, Conrads G, Richter EJ. Microbial leak-

age and marginal fit of the implant-abutment interface. Int J Oral Maxillofac Implants 1997; 12: 527-540.

16) Gross M, Abramovich I, Weiss EI. Microleakage at the abutment-implant interface of osseointegrated implants: a comparative study. Int J Oral Maxillofac Implants 1999; 14: 94-100.

17) Tesmer M, Wallet S, Koutouzis T, Lundgren T. Bacterial colonization of the dental implant fixture-abutment interface: an in vitro study. J Periodontol 2009; 80: 1991-1997.

18) Steinebrunner L, Wolfart S, Bössmann K, Kern M. In vitro evaluation of bacterial leakage along the implant-abutment interface of different implant systems. Int J Oral Maxillofac Implants 2005; 20: 875-881.

19) Do Nascimento C, Pedrazzi V, Miani PK, Moreira LD, de Albuquerque RF Jr. Influence of repeated screw tightening on bacterial leakage along the implant-abutment interface. Clin Oral Implants Res 2009; 20: 1394-1397.

20) Koutouzis T, Mesia R, Calderon N, Wong F, Wallet S. The effect of dynamic loading on bacterial colonization of the dental implant fixture-abutment interface: an in-vitro study. J Oral Implantol 2014; 40: 432-437.

21) Kitagawa T, Tanimoto Y, Odaki M, Nemoto K, Aida M. Influence of implant/abutment joint designs on abutment screw loosening in a dental implantsystem. J Biomed Mater Res B Appl Biomater 2005; 75: 457-463.

22) Cosyn J, Van Aelst L, Collaert B, Persson GR, De Bruyn H. The peri-implant sulcus compared with internal implant and suprastructure components: a microbiological analysis. Clin Implant Dent Relat Res 2011; 13: 286-295.

23) Broggini N, McManus LM, Hermann JS, Medina RU, Oates TW, Schenk RK, Buser D, Mellonig JT, Cochran DL. Persistent acute inflammation at the implant-abutment interface. J Dent Res 2003; 82: 232-237.

24) Rangert B, Jemt T, Jorneus L. Forces and moment on Branemark implants. Int J Oral Maxillofac Implants 1989; 4: 241-247.

25) McCartney J. Intraoral connection of individual abutment attachments for osseointegrated implant-supported prosthesis. J Prosthet Dent 1991; 66: 799-803.

26) May Kb, Edge MJ, Russell MM, Razzoog ME, Lang BR. The precision of fit at the implant prosthodontic interface. J Prosthet Dent 1997; 77: 497-502.

27) Dibart S, Warbington M, Su MF, Skobe Z. *In vitro* evaluation of bacterial leakage along implant-abutment interface of different implant systems. Int J Oral Maxillofac Implants 2005; 20: 875-881.

28) Besimo CE, Guindy JS, Lewetag D, Meyer J. Prevention of bacterial leakage into and from prefabricated screw-retained crowns on implants in vitro. Int J Oral Maxillofac Implants 1999; 14: 654-660

29) Duarte AR, Rossetti PH, Rossetti LM, Torres SA, Bonachela WC. *In vitro* sealing ability of two materials at five different implant-abutment surfaces. J Periodontol 2006; 77: 1828-1832.

30) Passos SP, Gressler May L, Faria R, Özcan M, Bottino MA. Implant-abutment gap versus microbial colonization: clinical significance based on a literature review. J Biomed Mater Res B Appl Biomater 2013; 101: 1321-1328.

31) Hermann JS, Schoolfield JD, Nummikoski PV, Cochran DL. Crestal bone changes around titanium implants. A histometric evaluation of unloaded non submerged and submerged implants in the canine mandible. J Periodontol 2000; 71: 1412-1424.

32) Tonetti MS, Schmid J. Pathogenesis of implant failures. Periodontol 2000 1994; 4: 127-138.

33) Schmitt CM, Nogueira-Filho G, Tenenbaum HC, Lai JY, Brito C, Döring H, Nonhoff J. Performance of conical abutment (Morse Taper) connection implants: a systematic review. J Biomed Mater Res A 2014; 102: 552-574.