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A randomized clinical trial about presence of pathogenic microflora and risk of peri-implantitis: comparison of two different types of implant-abutment connections

F. MENCIO, F. DE ANGELIS, P. PAPI, D. ROSELLA, G. POMPA, S. DI CARLO

Department of Oral and Maxillofacial Sciences Implant-Prosthetic Unit, "Sapienza" University of Rome, Italy

Abstract. – OBJECTIVE: The aim of this *in vivo* study was to evaluate two different types of implant-abutment connections: screwed connection and cemented connection, analyzing peri-implant bacteria microflora as well as other clinical parameters.

PATIENTS AND METHODS: Twenty implants were selected, inserted in 20 patients, 10 with a screwed implant-abutment connection (Group 1) and 10 with a cemented implant-abutment connection (Group 2). The peri-implant microflora was collected, after at least 360 days from the prosthetic rehabilitation, using paper points inserted in peri-implant sulcus for 30 s. Polymerase chain reaction (PCR) Real-time analyzed the presence of 9 bacteria periodontal-pathogens and *Candida albicans*.

RESULTS: Our findings showed that bacteria colonized all Groups analyzed, the average bacterial count was 3.7 E +08 (±1.19) in Group 1, compared to 2.1 E +08 (±0.16) in Group 2; no statistically significant differences were observed ($p > 0.0.5$). In Group 1, however, bacterial colonization of peri-implant sulci was over the pathogenic threshold for 5 bacteria, indicating a high-risk of peri-implantitis. Also in Group 2, results showed a microflora composed by all bacteria analyzed but, in this case, bacterial colonization of peri-implant sulci was over the pathogenic threshold for only 1 bacterium, indicating a lower risk of peri-implantitis. Moreover, clinical parameters (PPD > 3 mm and m SBI > 0) confirmed a greater risk of peri-implantitis in Group 1 compared to Group 2 ($p < 0.05$).

CONCLUSIONS: We concluded that, also after only 360 days, implants with screwed connection showed a higher risk of peri-implantitis that implants with cemented connection.

Key Words
Implant-abutment connection, Screwed connection, Cemented connection, Microbiological leakage, Peri-implantitis.

Introduction

Nowadays, dental implant surgery is a widely used procedure to replace missing teeth; over the years improvements in design, surface and surgical protocols have made implants a secure and highly predictable procedure with a mean survival rate of 94.6% and mean success rate of 89.7% after more than 10 years[1]. Dental implant failures are essentially mechanical or biological, and they are classified on a chronological basis as: a) early complications, resulting from surgical trauma, inadequate bone volume, lack of primary stability, bone infection or bacterial contamination; b) late complications, related to microbiological (peri-implantitis) and biomechanical changes (occlusal overload)[2]. Dental implant systems consist of two main parts: the abutment and the implant body. The implant-abutment connection represents the weakest point of the dental implant, due to a micro-gap between the implant-abutment interface, which may cause microbial leakage. These hollow spaces may act as reservoir for commensal and/or pathogenic bacteria, especially anaerobic or micro-aerophilic species, representing a potential source of tissue inflammation, which may lead to bone resorption[3-6]. Several Researches[7,8] have shown that either micro-movements or bacterial leakage may be considered as triggers factors for peri-implantitis. Sixth European Workshop in Periodontics of 2008 defined peri-implant diseases as: a) mucositis is the presence of inflammation of the peri-implant mucosa without signs of loss of bone support; b) peri-implantitis is characterized by a loss of bone support, in addiction to inflammation of the mucosa[9]. Microbiota associated to peri-implantitis are very similar to periodontal diseases, such as the species of the red and orange complexes, *Prevotella nigrescens*,

Corresponding Author: Piero Papi, DDS; e-mail: piero.papi@uniroma1.it

Campylobacter rectus and *Aggregatibacter actinomycetemcomitans*, as well as *Staphylococcus aureus*, *Enteric bacilli* and *Candida albicans*[10,11]. Increases in pocket depth and bone loss are associated with significant changes in the composition of sub-gingival microbiota, including: an increase of total bacteria load, with higher values of *Aggregatibacter actinomycetemcomitans*, *Fusobacterium species*, *Prevotella intermedia* and *Porphyromonas gingivalis*[12]. The aim of this study was to evaluate peri-implant bacteria microflora in two different types of implant-abutment connection systems, after at least 360 days of prosthetic loading. The authors hypothesized that screwed-retained connections may show higher values of total bacterial count.

Patients and Methods

Study Design

To address the research purpose, the authors designed and implemented a randomized clinical trial, conducted at the Department of Oral and Maxillo-Facial Sciences, at "Sapienza" University of Rome. The study was approved by the local Ethic Committee (Ref. 3691). The study sample was composed of a population of subjects presenting at the University's Department for Implant Treatment of the Premolar/Molar Region. To be included in the study sample, patients had to meet specific inclusion and exclusion criteria (Table I).

Table I. Inclusion and exclusion criteria.

Inclusion criteria
• Age >18 years
• Agreed to be included in the study and signed informed consent form
• Need of implant treatment in premolar/molar region
• FMPS < 25% and FMBS < 25%
• Absence of bleeding on gentle probing (<0.25N), PPD <5 mm and absence of radiographic bone loss assessed in paralleled periapical radiographs (Lang & Berglundh 2011)
Exclusion criteria
• Uncontrolled systemic diseases
• Use of drugs (antibiotics, anti-inflammatory and corticosteroids) in the previous 3 months
• Untreated acute oral diseases (caries, endodontic lesions, periodontal disease)
• Surgical treatment for peri-implantitis or periodontal disease in the previous 6 months
• Bruxism or clenching parafunctional habits
• Smokers (>10 cigarettes/day)
• Pregnant or lactating patients
• History of mental disorders

Study Variables

The binary predictor variable was implant-abutment connection and included screwed retained implant-abutment connection or cemented implant-abutment connection. The primary outcome variable was Total Bacterial Count (TBC), measured by quantitative Real-time PCR. Secondary variables included specific bacterial counts for 10 microbiota: *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); Candida Albicans (Ca).*

Other variables recorded included clinical and radiological peri-implant parameters:

- PPD (Probing Pocket Depth);
- m SBI (Modified Sulcus Bleeding Index) (Mombelli et al 1987)[13];
- m GI (Modified Gingival Index) (Mombelli et al 1987)[13];
- m PI (Modified Plaque Index) (Mombelli et al 1987)[13];
- REC (Gingival Recession);
- Peri-implant bone loss.

Surgical and Prosthetic phases

Patients were divided into two groups based on implant-abutment connection type: implant with screwed implant-abutment connection (Group 1) and implant with cemented implant-abutment connection (Group 2). Randomization for implant-abutment connection type was performed by simple allocation: a total of 10 patients were treated for each group. Two different implant systems were used: in Group 1 were placed implants with a machined surface made of commercially pure titanium grade 4; in Group 2 implants with surfaces sanded with aluminum oxide medium grade particles (250 μm) and etched with a solution of hydrofluoric acid at 3% and nitric acid at 30%, with a fixture›s core made of commercially pure titanium grade 4. Two experienced surgeons (FM, PP) performed all surgical procedures, following the same surgical protocol (Table II). Prosthetic rehabilitation was performed in an interval of time between 3 or 4 months after surgical treatment. Total thickness flaps were performed to discover implants and healing collars were placed for 14 days, before impression taking. Subsequently, implants were rehabilitated using metal-ceramic crowns: only single crowns were performed. For Group 1, the abutment was

Table II. Surgical protocol.

- Alginate impression for diagnostic study model
- Tc Dentascan
- Diagnostic wax-up
- Surgical template
- Supra-gingival debridement and oral hygiene instructions
- Local anesthesia with adrenaline 1:100.00
- Rinse with chlorexidine 0.2% for 60 s
- Total thickness flap, preserving periodontium of adjacent teeth
- Detachment of muco-periosteal flap
- Preparation of implant site with progressive diameter drill, following manufacturer's recommendations
- Control with paralleled periapical radiographs
- Implant placement with submerged technique
- Silk sutures 3-0
- Post-surgical instruction (antibiotic, anti-inflammatory, corticosteroids)

inserted into the implant and jointed with connection screw using the specific screwdriver and then the dynamometric ratchet, tightening the screw to 25 Ncm as per manufacturer specification. For Group 2, the collar was inserted inside the fixture using a specific device. The abutment, then, was cemented with Panavia 2.1 (J. Morita, Irvine, CA, USA). The cement was mixed according to manufacturer's recommendations and applied on the axial surface of the internal portion of the implant to minimize hydrostatic pressure during sealing. Abutments were cemented on the implants with load of 5 kg maintained for 10 min. The cement in excess was removed with a scaler. Two expert investigators performed all procedures (FDS, DR). After screwing or cementing implant-abutment connection, crowns were cemented on the implant with a non-eugenol zinc oxide provisional cement (TempBond NE, Kerr Dental, Orange, CA, USA). The cement in excess was removed with an ultrasonic scaler. Patients were then included in a professional hygiene maintenance protocol every three months.

Data collection

One implant for each patient was randomly selected to perform the microbiological assessment, clinical and radiological peri-implant measurements, as well as to analyze cumulative implant survival rate using the Kaplan-Meier survival analysis. After 12 months of prosthetic loading, bacteria collection was performed. Samples were obtained from the peri-implant sulcus in 6 sites around each implant: Buccal, Mesio-Buccal, Disto-Buccal, Palatal, Mesio-Palatal, Disto-Palatal. Sampling was performed using sterile absorbent paper tips inserted into the peri-implant sulcus for 30 s. Prior to subgingival plaque sampling, supragingival plaque was removed from implants and neighboring teeth using Teflon curettes Implacare (Hu-Friedy Mfg. Co., LLC, Chicago, IL, USA) without penetrating the gingival or peri-implant sulcus. Cotton rolls were used for relative isolation and sampling sites were dried with air. The paper tips, with the samples, were placed into the Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and were sent for microbiological analysis to the laboratory Institut Clinident SAS (Aix-en-Provence, France) in the provided mailing envelopes. After the sampling, a clinical exam was performed in 6 sites around implant using a millimetric probe HAWE CLICK-PROBE (Hawe Neos Dental, Bioggio, Switzerland) and PPD was recorded. As for m SBI, m GI and m PI, they were evaluated in 4 sites around implant and the mean value was calculated getting implant's score. Presence or absence of REC was evaluated. A standardized periapical radiograph (Rinn, Dentsply, York, PA, USA) was taken for each implant to evaluate peri-implant bone loss levels.

Quantitative Real-time PCR assays

Quantitative real-time PCR was carried out for 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 more *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, 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 were derived from previously published ribosomal 16 S sequences[14,15] and were adapted to Real-time PCR conditions. Assays were carried out on the RotorGene® Q thermal cycling system (Qiagen, Hilden, 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 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, Hilden, Germany). Serial dilutions of bacterial standard DNA provided by Institut Clinident SAS 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

At the end of study all collection data were inserted in a work sheet (Microsoft Excel, Microsoft Corporation, Redmond, WA, USA) in a personal computer (Macbook Pro, Apple, Cupertino, CA, USA) and analyzed using a specific software (IBM SPSS V10 Statistics, IBM, Armonk, USA). Differences in the bacterial counts from two groups were compared using the Mann-Whitney *U* test. A p-value <0.05 was considered significant.

Results

A total of 20 patients were selected, 10 male and 10 female, with a mean age of 46.67 years (range= 27-62 years). A total of 50 implants were placed, 25 for each group (Table III). The follow-up ranged between 360 days and 2 years with a mean follow-up of 484 days, approximately 1 years and 4 months (Table IV). Three upper right first molars, six upper left first molars, two lower left first molars and nine lower right first molars were included in this study. All peri-implant sulci showed a bacterial colonization, but in Group 1 presence of bacteria showed higher values compared to Group 2. The average total bacterial count was 3.7 E+08 (±1.19) for Group 1, compared to 2.1 E+08 (±0.16) in Group 2, without statistically significant differences (p=0.32). Group 1 showed a microflora composed by all bacteria analyzed, excluding *Aggregatibacter actinomvcetemcomitans* and *Candida albicans.*

Table III. Patient's demographics and treatment sites.

N°	Name	Gender	Age	Edentulous Area
1	BD	F	44	3.6-4.7
2	PR	M	53	1.5-1.6-3.6-4.6
3	PL	F	47	1.6-2.6-4.4
4	RB	F	29	4.5-4.6
5	BM	M	50	1.4-4.6
6	SA	M	39	2.4-2.6
7	DV	F	57	2.5-2.6-4.6
8	DG	F	33	1.5-1.6
9	MV	M	48	1.4-4.6
10	PS	F	37	2.4-2.5-2.6
11	BA	M	61	1.5-1.6-2.4
12	CM	F	38	4.6
13	ZA	M	44	2.4-2.5-2.6-2.7
14	ST	F	47	3.6-4.6
15	LM	F	49	3.5-4.6
16	MS	M	56	2.5-2.6
17	PA	F	46	1.5-3.6
18	AN	M	27	2.5-4.5-4.6
19	JGD	M	45	2.5-4.6-4.7
20	RG	M	62	1.4-1.5-1.6

Table IV. Study design and follow-up. The red sites are implants included in the study sample.

N°	Group 1 (screwed implant-abutment connection)			Group 2 (cemented implant abutment-connection)		
	Name	Follow-up	Implant Site	Name	Follow-up	Implant Site
1	BD	710 days	3.6-4.7	BA	537 days	1.5-1.6-2.4
2	PR	382 days	1.5-1.6-3.6-4.6	CM	504 days	4.6
3	PL	410 days	1.6-2.6-3.6	ZA	479 days	2.4-2.5-2.6-2.7
4	RB	378 days	4.5-4.6	ST	516 days	3.6-4.6
5	BM	546 days	1.4-4.6	LM	488 days	3.5-4.6
6	SA	625 days	2.4-2.6	MS	689 days	2.5-2.6
7	DV	630 days	2.5-2.6-4.6	PA	716 days	1.5-3.6
8	DG	426 days	1.5-1.6	AN	372 days	2.5-4.5-4.6
9	MV	398 days	1.4-4.6	JGD	412 days	2.5-4.6-4.7
10	PS	362 days	2.4-2.5-2.6	RG	381 days	1.4-1.5-1.6

Bacteria	Pathogenic load*	Pathogenic threshold**	Status***	% / Total Bacterial Count****
Aggregatibacter actinomycetemcomitans	0,0E+00	1,0E+03	-	0,00
Porphyromonas gingivalis	7,0E+06	1,0E+05	+++	1,92
Tannerella forsythia	5,9E+05	1,0E+05	++	0,16
Treponema denticola	2,1E+05	1,0E+05	++	0,06
Prevotella intermedia	1,3E+06	1,0E+05	+++	0,36
Parvimonas micra	2,6E+05	1,0E+06	+	0,07
Fusobacterium nucleatum	1,9E+06	1,0E+07	+	0,51
Campylobacter rectus	1,6E+06	1,0E+06	++	0,43
Eikenella corrodens	4,5E+05	1,0E+07	+	0,12
Candida albicans	0,0E+00	N/A	**Negative**	N/A
Total Bacterial Count	3,7E+08			

Figure 1. Group 1, screwed implant-abutment connection, average results. **Pathogenic load:* the amount of detected bacteria in 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 bacterial versus total bacterial count; N/A not available.

The bacterial colonization of peri-implant sulci was over the pathogenic threshold for 5 bacteria (*Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Prevotella intermedia, Campylobacter rectus*) indicating a high-risk of peri-implantitis (Figure 1). Also Group 2 showed a microflora composed by all bacteria analyzed, excluding *Aggregatibacter actinomycetemcomitans*. In this case, the bacterial colonization of peri-implant sulci was over the pathogenic threshold for only 1 bacterium (*Prevotella intermedia)*, indicating a low-risk of peri-implantitis. A patient presented *Candida albicans* disease (Figure 2, Figure 3). Clinical examinations showed different

Bacteria	Pathogenic load*	Pathogenic threshold**	Status***	% / Total Bacterial Count****
Aggregatibacter actinomycetemcomitans	0,0E+00	1,0E+03	-	0,00
Porphyromonas gingivalis	6,7E+04	1,0E+05	+	0,03
Tannerella forsythia	9,9E+03	1,0E+05	+	0,005
Treponema denticola	3,3E+03	1,0E+05	+	0,002
Prevotella intermedia	1,3E+06	1,0E+05	+++	0,61
Parvimonas micra	6,2E+04	1,0E+06	+	0,03
Fusobacterium nucleatum	5,7E+05	1,0E+07	+	0,28
Campylobacter rectus	3,0E+05	1,0E+06	+	0,14
Eikenella corrodens	1,1E+05	1,0E+07	+	0,05
Candida albicans	1,3E+04	N/A	**Positive**	N/A
Total Bacterial Count	2,1E+08			

Figure 2. Group 2, cemented implant-abutment connection, average results. **Pathogenic load:* the amount of detected bacteria in 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 Coun*t: relative proportion of a specific bacterial versus total bacterial count; N/A not available.

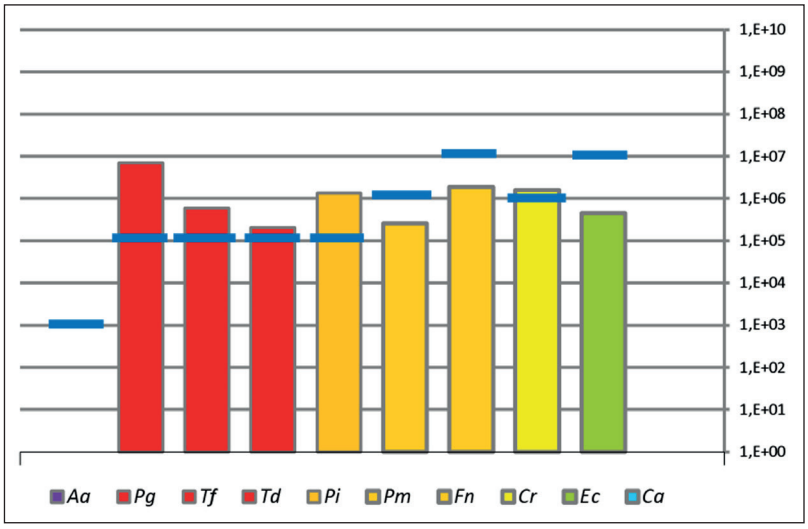

Figure 3. Group 1bacterial leakage, average results. Aa: *Aggregatibacter actinomycetencomitans*; Pg: *Porphyromonas gingivalis*; Tf: *Tannerella forsythia*; Td: *Treponema denticola*; Pi: *Prevotella intermedia*; Pm: *Parvimonas micra*; Fn: *Fusobacterium nucleatum*; Cr: *Campylobacter rectus*; Ec: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

results for implants with screwed implant-abutment connection and implants with cemented implant-abutment connection (Tables V, VI). In Group 1, fourteen sites showed PPD values of more than 3 mm (23%), with an average probing of 3.1 mm. In Group 2, only 2 sites reported values higher than 3 mm (3%) with an average probing of 1.8 mm (p=0.0013; p<0.05, X^2 test). Bleeding at probing was presented in 8 implants in Group 1 (80%) and in 6 in Group 2 (60%) (p=0.04; p<0.05, X^2 test). Gingival alterations were evaluated in 6 cases (60%) in Group 1 and in only 2 cases (20%) in Group 2. Mechanical failures were reported in two cases (20%) in Group 1, identified as abutment loosening, and in one case for Group 2, identified as abutment decementation. Radiographic alterations were noticed in two implants for Group 1 and in one implant for Group 2, but without signs of severe pathogenicity.

Discussion

In this study, a higher risk of developing peri-implantitis was found in Group 1, associated with a major presence of pathogenic bacteria of the "red" complex over the pathogenic threshold (Figures 4 and 5). Moreover, clinical examination data confirmed the hypothesis about a correlation between connection type and presence of periodontal pathogenic bacteria. Nowadays, frequencies of peri-implant diseases, such as mucositis and peri-implantitis, are respectively 54-80% and 28-56% for patients and 32-50% and 12-43% for

Table V. Results of clinical examinations in Group 1.

N°	Name	Site	PPD						mSBI	mGI	mPI	REC	Mf	Rx
			B	MB	DB	P	MP	DP						
1	BD	3.6	3	3	3	2	4	3	1	2	0	+	+	+
2	PR	4.6	5	3	6	2	2	3	2	2	1	+	-	-
3	PL	2.6	3	3	4	3	3	4	1	1	1	+	+	-
4	RB	4.6	2	2	3	2	3	2	0	0	1	-	-	-
5	BM	4.6	3	3	2	3	4	2	1	0	0	-	-	-
6	SA	2.6	2	2	4	3	2	2	1	0	2	-	-	-
7	DV	2.6	4	5	6	3	3	3	2	2	2	+	+	+
8	DG	1.6	3	4	3	3	4	3	1	0	1	-	-	-
9	MV	4.6	3	2	3	3	2	3	0	1	1	-	-	-
10	PS	2.6	3	4	5	3	3	3	1	1	0	-	-	-

PPD: probing pocket depth, the red numbers are the probing > 3 mm; m SBI: modified Sulcus Bleeding Index, 0- absent of bleeding, 1- bleeding to isolate spot, 2- linear bleeding, 3- spontaneus and profuse bleeding; m GI: modified Gingival Index, 0- normal mucosa, 1- edema, 2- edematous and polishes mucosa, 3- marked redness, edema, spontaneous bleeding; m PI: modified Plaque Index, 0- absence of plaque, 1- plaque detectable with probe, 2- visible plaque, 3- presence of abundant plaque deposits; REC: gingival recession, + present, - absent; Mf: mechanical failure, + present, - absent; Rx: radiographic alterations, + present, - absent.

Table VI. Results of clinical examinations in Group 2.

N°	Name	Site	PPD						mSBI	mGI	mPI	REC	Mf	Rx
			B	MB	DB	P	MP	DP						
1	BA	1.6	3	3	3	4	4	3	2	0	1	-	-	+
2	CM	4.6	1	2	2	1	2	2	0	0	0	-	+	-
3	ZA	2.6	2	2	2	1	2	2	1	0	1	-	-	-
4	ST	4.6	1	1	1	1	1	2	0	0	0	-	-	-
5	LM	4.6	1	2	2	1	2	2	1	0	1	-	-	-
6	MS	2.6	3	2	3	3	3	3	1	0	2	-	-	-
7	PA	3.6	1	2	1	1	1	2	0	0	0	-	-	-
8	AN	4.6	1	1	2	1	2	1	1	1	2	-	-	-
9	JGD	4.6	2	3	1	2	1	1	0	0	1	-	-	-
10	RG	1.6	2	3	3	2	3	3	1	1	2	-	-	-

PPD: probing pocket depth, the red numbers are the probing > 3 mm; m SBI: modified Sulcus Bleeding Index, 0- absent of beeding, 1- bleeding to isolate spot, 2- linear bleeding, 3- spontaneus and profuse bleeding; m GI: modified Gingival Index, 0- normal mucosa, 1- edema, 2- edematous and polishes mucosa, 3- marked redness, edema, spontaneous bleeding; m PI: modified Plaque Index, 0- absence of plaque, 1- plaque detectable with probe, 2- visible plaque, 3- presence of abundant plaque deposits; REC: gingival recession, + present, - absent; Mf: mechanical failure, + present, - absent; Rx: radiographic alterations, + present, - absent.

implants[16,17]. The presence of a pathogenic microflora in a initial phase can be associated with an higher risk of peri-implantitis, as per periodontal diseases[18,19]. In 1992, Socransky and Haffajec[20] modified the postulates of Koch establishing criteria that identify periodontal pathogens. The study of biofilm using DNA hybridization techniques made possible to individuate specific bacterial species and relate them to clinical health or disease. While the "purple" (with fundamentally aerobic flora lacking mobility), "yellow" and "green" complexes are not associated to disease, the "orange" (F. *nucleatum, P. intermedia, P. micros*) and "red" complexes (*P. gingivalis, T. forsythia, T. denticola*) are disease-related. *A. actinomycetemcomitans* is also considered as being periodontopathogenic, although it is not included in any group. Factors influencing disease include susceptibility of individual host, presence of interacting bacterial species and local environment of periodontal pocket. Several authors[21-23] compared microbiota founded in dental implants classified as being healthy with implants with a diagnosis of peri-implantitis (*A. actinomycetemcomitans)* resulted the predominant pathogen implicated in peri-implant destruction. Finally, an important risk factor for peri-implant disease may be type of implant abutment-connection, associated to dental implant position with respect to alveolar bone crest[24]. The presence of a micro-gap between implant and abutment (estimated between 20 and 49 micron) allows passage of bacteria from peri-implant sulcus to the internal part of implant (in particular for screwed-implant)[25]. This environment

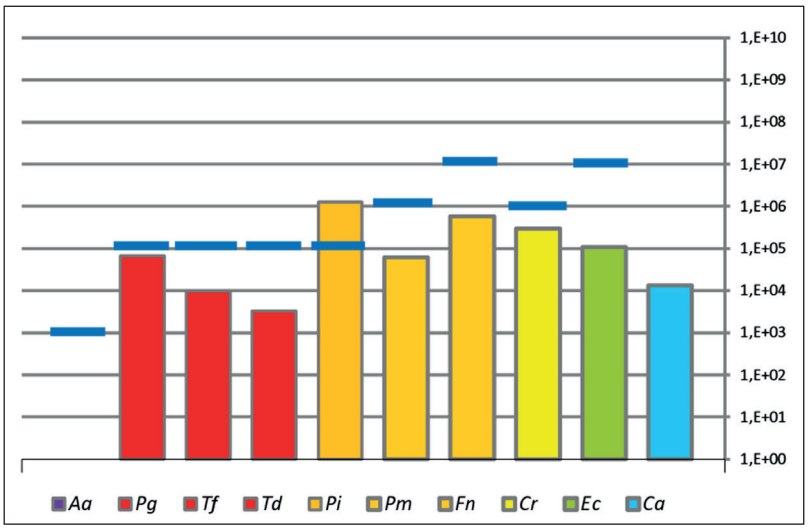

Figure 4. Group 2 bacterial leakage, average results. Aa: *Aggregatibacter actinomycetencomitans*; Pg: *Porphyromonas gingivalis*; Tf: *Tannerella forsythia*; Td: *Treponema denticola*; Pi: *Prevotella intermedia*; Pm: *Parvimonas micra*; Fn: *Fusobacterium nucleatum*; Cr: *Campylobacter rectus*; Ec: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

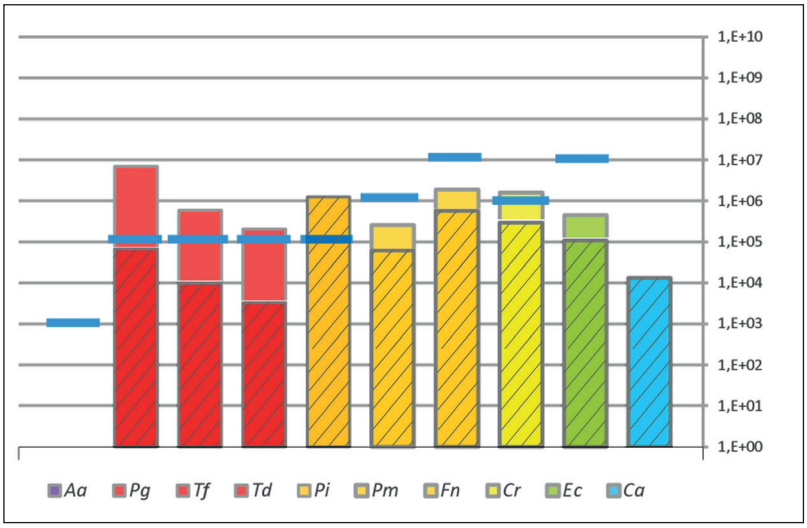

Figure 5. Bacterial colonization Group 1 and Group 2. Graph of comparison. Overlapping rectangles of the histogram. Dashed rectangles represent results of Group 2.

is more favorable for bacterial growth, especially anaerobic bacteria (such as most important parodonto-patogenes)[26-28]. According to the authors, further research in the field of microbiological peri-implant leakage, with long-term follow-up studies, larger sample and different implant-abutment connections, are necessaries to verify the hypothesis presented by this study.

Conclusions

According to our findings, even after a maximum of 2 years of functional loading, peri-implant sulcus of both groups presented a colonization by "red complex" bacteria and a higher risk to develop peri-implant diseases, confirmed by clinical parameters. This risk was higher in Group 1 compared to Group 2, due to the major presence of bacteria associated with peri-implantitis over the pathogenic threshold. Within the limitations of this study, it is possible to conclude that cemented-tapered connection is more impermeable that screwed-connection, that could be a reservoir of dangerous bacteria.

Conflict of Interest

The authors declare they have no conflict of interest.

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