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"IMPLANT- ABUTMENT INTERFACE: BACTERIAL LEAKAGE AND MICROGAP FORMATION IN TWO DIFFERENT TYPES OF IMPLANT-ABUTMENT CONNECTIONS"

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If you can talk with crowds and keep your virtue,
Or walk with Kings—nor lose the common touch,
If neither foes nor loving friends can hurt you,
If all men count with you, but none too much:
If you can fill the unforgiving minute
With sixty seconds' worth of distance run,
Yours is the Earth and everything that's in it,
And—which is more—you'll be a Man, my son!

Per voi.
Per te mamma.
Per te papà.
Grazie

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ABSTRACT

Aim

The aim of this study is to evaluate, both *in vitro* and *in vivo*, two different types of implant-abutment connections: screwed connection and cemented connection, analyzing the permeability of the IAI to bacterial colonization and the stability to chewing forces.

Materials and Methods

In this study were compared two different types of implant-abutment connections: internal hexagon screwed connection (Winsix®, BioSAF IN, Ancona, Italy) Group 1; and internal hexagon cemented-conical connection (Bone System®, Milano, Italy) Group 2. Group 1 and Group 2 were compared on three levels: impermeability to bacterial penetration an *in vitro* study, resistance to loading for 5 years a *simulated computer model*, type of peri-implant bacterial colonization and health of peri-implant soft tissues around the implant for 2 year in an *in vivo* study.

Results

The results had showed the lower stability to the screwed implant-abutment connection than the cemented implant-abutment connection both for the permeability to the bacterial colonization than for the stability to the chewing forces.

Conclusion

Also if the implants long-term failures are consequences of multi-factorial elements the choice of an adequate implant system is fundamental for the long-term success. Also the choice of connection system is very important and would be preferable to choose implants with cemented connection instead of implants with screwed connection.

1. INTRODUCTION

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

Elevated success rates has been described in the current literature on the long-term treatment with osseointegrated dental implants.[2-4]

Usually, these rates have been referred to the immediate stabilization process, mainly associated to the quality and characteristics of the implant used.[5]

Primary stability of the implant has been reported as the key factor to initialize implant survival.

In spite of the excellent success rates in osseointegrated implant rehabilitations, failures have been described in the literature, and related to mechanical and microbiological factors, frequently acting in association.[6-7]

The unfavourable occlusion and diversity of microorganisms harbouring in 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 dental endosseous implant fixtures, as it must resist maximal and permanent chewing forces as well as penetration by bacteria.

Two-piece implant unavoidably present a micro-gap between the implant and the abutment.

In fact, when a prosthetic abutment is connected to a fixture, a micro-gap is created between the components, caused by 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 facing the fixture-abutment junction.[10-12]

This inadequate fit between the implant and the abutment may be considered a risk factor similar to that in poorly adapted dental restorations, capable of leading to clinical and microbiological alterations in the peri-implant tissues. Furthermore, because, of allow micro-movements of the abutment, a lack of fit between it and the implant presents a biomechanical risk, since it enables the set to be submitted to undesirable loads, capable of resulting in loosening of fracturing the prosthetic screw, or fracturing the implant body.[13]

Callan et al, analyzing the size of the fixture abutment interface, found mean values between 30 µm and 135 µm [14]; Dellow et al between 0 and 7.15 µm [15]; and Jansen between 1 and 10 µm.[16] Due to the existence of the interface, the possibility of fluid and microorganism exchanges is very evident.

The implant abutment interface can allow the passage of fluids and bacteria, irrespective of the implant system (with tapered of flat connections). Even well fitting interfaces (smaller than 5 µm) were incapable of preventing bacterial leakage and colonization of internal implant surface. A large variety of microorganisms appears 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* present 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 interface with maladjustment within the values described in the literature.[16]

Jansen et al compared the size of the spaces and proportion of contamination. They found no statistically significant correlation between the size of these spaces (means), determinate by scanning electronic microscopy, and the proportion of leakage, by means of the microbiologic test. Some implants were in accordance with this trend, while others that had a low maladjustment means obtained a high contamination index.[16]

Both *in vitro* and *in vivo* studies have demonstrated contamination of the internal portion of osseointegrable implants by bacteria and early bacterial colonization of implant surface and peri-implant tissue can occur within minutes after implant placement.[17]

The gap in IAI is found in all the implant system studied in the literature. Its clinical significance is frequently ignored by professionals that use the connectors without applying the due torque indicated by the manufacturer. The importance of the idea torque used on the screw that retains the prosthetic connector must be taken into consideration, since it could interfere in the size of the micro-space of the IAI.[18;19]

According to Weiss et al, the repeated removal and placement of the connectors while the prosthesis is being made could alter the surface of the screw threads and the internal parts of the implant, causing progressive loss of the recommended torque, favouring an increase in IAI.[19]

Moreover many studies have shoved that the presence of a fixture-abutment interface micro-gap in close relation to bone may this have a role in the development of peri-implant

inflammation and bone loss.[20-21] Therefore, long-term role of this bacterial colonization must be considered in the maintenance of healthy peri-implant tissues.[13]

A recent systematic review concluded that while the positioning of the micro-gap may influence crestal bone level changes, the impact of the implant-abutment connection lacks documentation.[22]

The potential colonization of the internal connection through the implant-abutment micro-gap is probably related to multifactorial 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 re-tightening; and the loading force when the implant are in function.[18;23-28]

Many in vitro studies have demonstrated bacterial leakage along the implant-abutment interface of several implant connection under unloaded conditions.[6;29-33]

In 2008 Do Nascimento et al. investigated leakage of *Fusobacterium nucleatum* through the interface between implant and premachined or cast abutments. Bacterial growth in the medium, indicative of microbial leakage, was found only in 2 out of 18 samples (11.1%).[30]

Barbosa et al., in a 2009 study, compared conventional bacterial culture and DNA Checkerboard hybridization method to evaluate bacterial leakage along the IAI. 6 implant-abutment assemblies out of 20 (30%) showed turbidity after 14 days.[32]

In 2009 Do Nascimento et al, *in vitro* study, demonstrated that bacterial penetration along the implant-abutment interface as a consequence of abutment screw loosening. The results of this study suggest that bacterial leakage between implants and abutments occurs even under unloaded conditions and at a higher intensity when the abutment screw is tightened and loosened repeatedly.[25]

Always Do Nascimento et al., in 2011, published a study where the aim was to compare the detection frequency of bacterial leakage from human saliva through the implant-abutment interface, using either DNA Checkerboard or culture method. Positive signals of bacterial leakage were showed in 6 of the 15 evaluated samples. *Capnocytophaga gingivalis* and *Streptococcus mutans* were the most frequently detected species harbouring the internal surface of the implants.[31]

These gaps can be further enlarged under loading when the implant assembly components are subjected to eccentric forces. Screw loosening and decrease in screw joint preload below a critical level may contribute to joint instability, causing clinical failure. Also

micro-movements of the implant components during function may allow the initiation of a pumping effect, causing bacteria to move through the implant-abutment interface.[34]

Steinebrunner et al demonstrated in vitro leakage of bacteria along this pathway as result of failure of adaptation of the components after a simulation of fatigue loading. The authors concluded that all the system that they evaluated showed bacterial leakage along the implant abutment interface, and that the number of load cycles until bacterial penetration occurred differed significantly between implant system and their specific connection designs.[24]

In a clinical investigation, Cosyn et al confirmed the occurrence of bacterial penetration through this interface.[28]

Kitagawa et al, in a 2005 study, investigated the influence of implant-abutment joint designs on abutment screw loosening, using nonlinear dynamic analysis of the finite element method (FEM) and demonstrated the difference in rotation of components in dental implant system with taper or external hex joints.[27]

Koutouzis et al, in an *in vitro* study, demonstrated that the geometry of the fixture-abutment interface influence the risk of bacterial invasion into the internal part of the implant under dynamic-loading conditions. 28 implants were divided into two groups (n=14 per group) based on their micro-gap dynamics. Group 1 was comprised of fixtures with internal Morse-taper connection that connected to standard abutments and group 2 was comprised of implants with a four-groove conical internal connection that connected to multibase abutments. The specimens were immersed in a bacterial solution of *Escherichia coli* and loaded with 500,000 cycles of 15 N in a wear simulator. The results showed that one of the 14 samples in group 1 and 12 of the 14 samples in group 2 developed multiple colony forming units for *E. Coli.* Moreover implants in group 1 exhibited an increase in torque value in contrast to implants in group 2, which exhibited a decrease. [26]

Different connection have been compared regarding their mechanical stability. Higher stability under loading conditions has been reported for different internal connections compared to external connection.[27;35]

According to Salvi & Lang (2001), under functional loading, the mechanical instability of the external connection promotes micro-movements of the abutment.

Differently from other connections, the Morse-cone connection locks the implant-abutment system because of the friction between the external wall of the abutment and internal wall of the implant.[36]

In 2012, Do Nascimento et al. published an in vitro study where were compared different implant connections under unloaded and loaded conditions. In this study were used three different implant connections: external-hexagon, internal-hexagon and Morse-cone. The samples were immersed in human saliva either in static conditions or loaded with 500,000 cycles at 120 N. The conclusions of this study suggest that bacterial species from human saliva may penetrate along the implant-abutment interface under both unloaded and loaded conditions for all connections evaluated. Morse-cone connection implant showed the lowest counts of microorganisms for both conditions. External- and internal-hex implants showed a higher incidence of bacteria and higher bacteria counts after simulated loading.[34]

A recent review of literature performed by Schmitt et al. in 2013 were compared conical versus non conical implant-abutment connection systems in terms of their *in vitro* and *in vivo* performances. 52 studies met inclusion criteria and were included in this systematic review. The results indicated that, *in vitro* studies, the conical and nonconical abutments showed sufficient resistance to maximal bending force and fatigue loading. However, conical abutments showed superiority in terms of seal performance, micro-gap formation, torque maintenance, and abutment stability. *In vivo* studies (human and animal) indicated that conical and non conical systems are comparable in terms of implant success and survival rates with less marginal bone loss around conical connection implants in most cases. This review indicates that implant system using a conical implant-abutment connection, provides better results in terms of abutment fit, stability, and seal performance.[37]

The evidence regarding differences in microbial penetration of the implant-abutment micro-gap with different connection designs is very limited and mostly based on *in vitro* studies. Many of these studies claim the conical connections have smaller gaps and thus are superior to other internal and external screw-retained connection in limiting bacterial leakage;[23;26;38;39] while other authors conclude that differences are non-significant.[40]

Therefore, consensus does not exist even within both *in vitro* and *in vivo* studies.

In any case, no endosseous dental implant system can currently provide a complete seal at the implant-abutment interface, occurring bacterial leakage irrespective of the type of connection.[13;22]

However, irrespectively of the complexity of models built to simulate chewing and the intraoral environment, reproducing *in vitro* clinical conditions remains impossible.

In fact, available comparative clinical studies have only analyzed success rate and bone level changes of implant with different connection. However, no clinical studies has been reported regarding bacterial permeability of different implant-abutment connections under *in vivo* functional conditions, and thus a lack of evidence remains.[41]

In a recent study published on Clinical Oral Implants Research, Canullo et al evaluated the bacteria micro-flora present inside the implant connection and in the peri-implant sulcus fluid of healthy implants, and analyzed the relationships between these harbouring sites for four different implant systems after at least 5 years of functional loading.

Regarding the analysis of positivity to bacteria in the peri-implant sulcus no significant differences were observed. Analyzing the connection's inside, none of the connection designs showed the capacity to prevent microbiological leakage through the implant-abutment micro-gap. Conical connection presented the lowest mean values for red complex bacteria and external hexagon connection the highest. Moreover internal hexagon with external collar connection and conical connection had significantly lower total bacterial counts in the peri-implant sulcus and inside the connection.

The authors concluded that the connection design might influence bacterial activity levels quantitatively and qualitatively, especially inside implant connection.[41]

In conclusion, several studies have demonstrated the presence of microgap between implant and abutment and the occurrence of bacterial leakage in IAI. However, artifices can be created to make the clinical significance of this gap negligible. The supracrestal position of 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 can present clinical significance. The occurrence of bacterial leakage at the internal surface of implants, through IAI, is one of the parameters for analyzing the degree of quality in fabrication of these connections.

In this contest the design of connection and the type of system used for to connected the implant and the abutment are two very important factors.

The aim of this study is to evaluate, both *in vitro* and *in vivo,* two different types of implant connection designs: cemented connection and screwed connection, analyzing IAI's permeability to bacterial colonization and the stability to chewing forces.

2. MATERIALS AND METHODS

Study design

In this study were compared two different types of implant-abutment connections: internal hexagon screwed connection (Winsix®, BioSAF IN, Ancona, Italy) Group 1 (Fig. 2.1); and internal hexagon cemented-conical connection (Bone System®, Milano, Italy) Group 2. (Fig.2.2)

The implant system of Group 1 presents an implant-abutment connection called Free Lock® with a specific design. It presents an internal hexagon and a joint between implant and abutment using a connection screw. The manufacturer claims that the internal length and the diameter of connection screw are significantly greater than the average (L.7mm and Ø 2mm), ensuring a high stability of the connection. The special design of internal Free Lock® geometry allows: an increase of the mechanical resistance to extra-axial load; an increase of the contact surface; the greater stability of the implant-abutment connection, decreasing the microcirculation of biological fluids inside; maximum stability of the superstructure. Careful and controlled production of manufactured certifies coupling tolerances within 10 micron, making the connection more precise and reproducible.[42]

The implant system of Group 2 presents an implant-abutment connection called Dual Retained Connection®. The specific element presented in this system is an intermediate component between implant and abutment: the trans-mucosal collar. Thanks to this device is possible an exclusive dual retention: mechanical and chemical. The first is possible using a pressure inserting to the collar in the implant (Morse taper connection) the second using the sealing of abutment inside the implant through the trans-mucosal collar. The manufacturer claims that the Dual Retained Connection® ensures a joint absolutely stable and totally devoid of micro-movements in time. Moreover the sealant effect of the cement completely deletes the possibility of bacterial penetration inside implant, preventing the formation of the reservoir that could compromise the health of soft tissue around the implant, making the connection absolutely impermeable to bacteria.[43]

In this study the Group 1 and Group 2 were compared on three levels: impermeability to bacterial penetration an *in vitro* study, resistance to loading for 5 years a *simulated computer model*, type of peri-implant bacterial colonization and health of peri-implant soft tissues around the implant for 2 year in an *in vivo* study.

Fig.2.1: *Group 1 Implant-Abutment assembly*

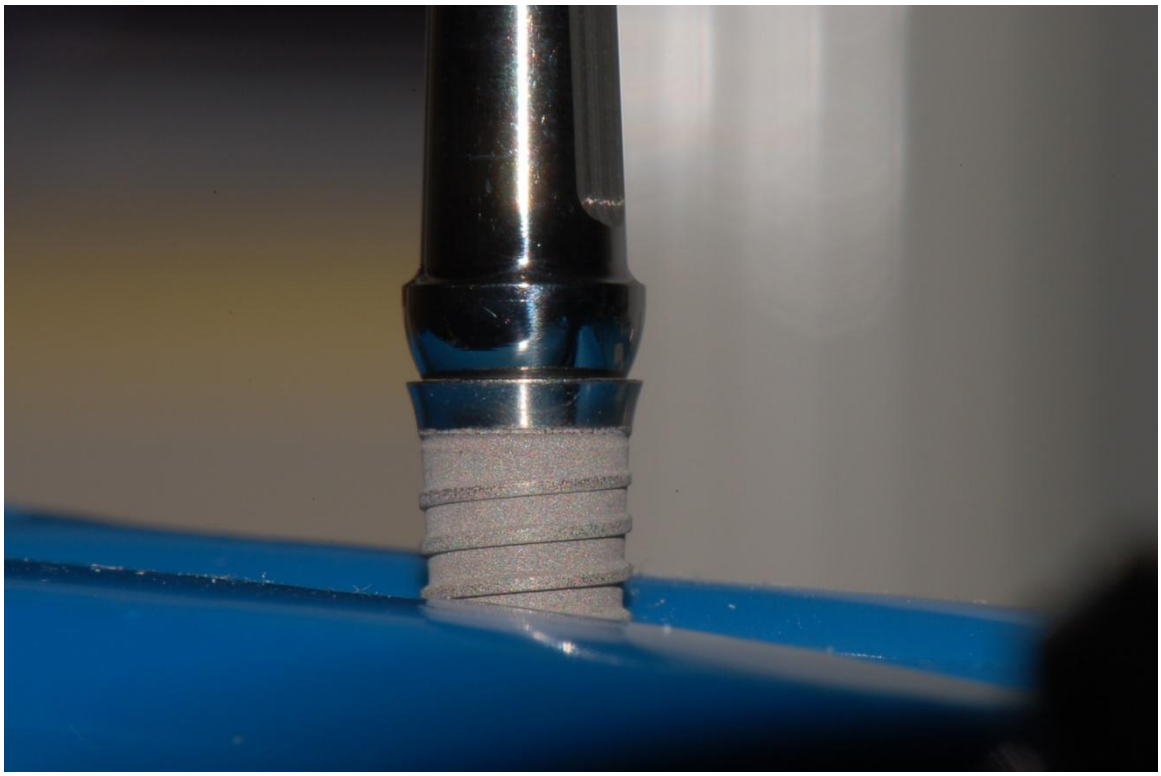


Fig. 2.2: *Group 2 Implant-transmucosal Collar-Abutment assembly*

2.1 Bacterial leakage of saliva in Implants with different implant-abutment connections: an *in vitro* study

Aim of the study

For this *in vitro* study, the two groups of implants were compared based on their impermeability to the bacterial colonization, evaluating the detection frequency of bacterial leakage from human saliva through the implant-abutment interface.

Study design

In this study a total of ten implants were tested, five in each experimental group: Group 1 consisted of fixtures (Winsix®, BioSAF IN, Ancona, Italy), 4.5mm in diameter (Fig. 2.1.1), with internal hexagon connected to the abutment with a retained screw.(Fig. 2.1.2; Fig. 2.1.3)

Fig. 2.1.1: *Group 1 implants dimensions.*

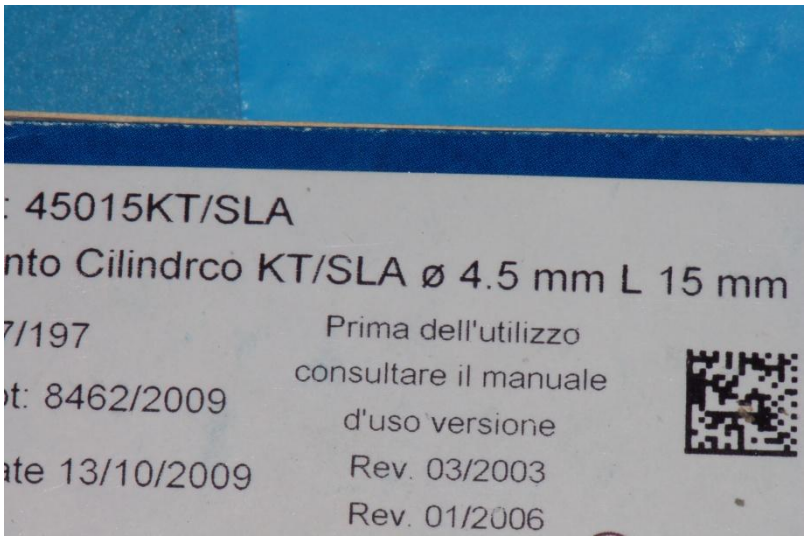


Fig. 2.1.2: *Implant Group 1*

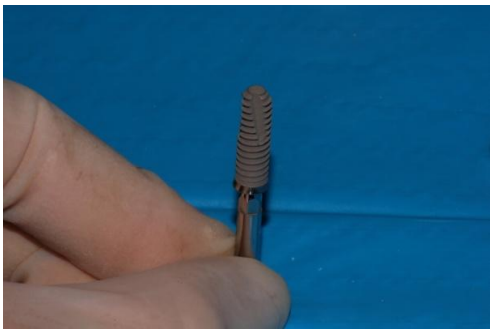


Fig. 2.1.3: *Abutment and retained screw*

The abutments were connected to the fixtures with a torque of 25 Ncm, according to manufacturer's protocol. (Fig. 2.1.4A-B; Fig.2.1.5 A-B)

Fig. 2.1.4 A-B: *Screw insertion*

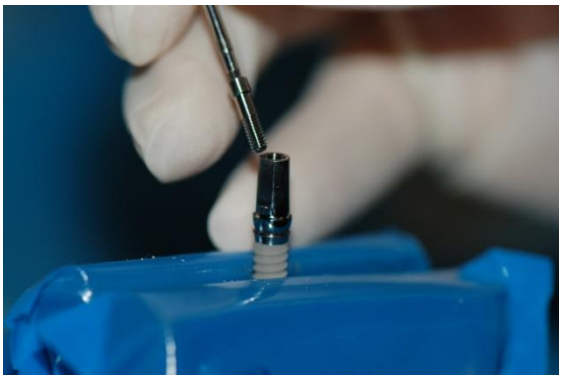
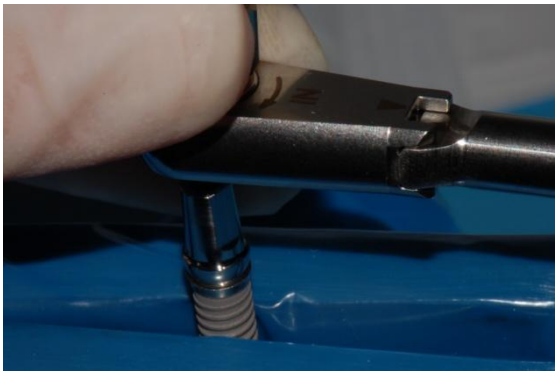
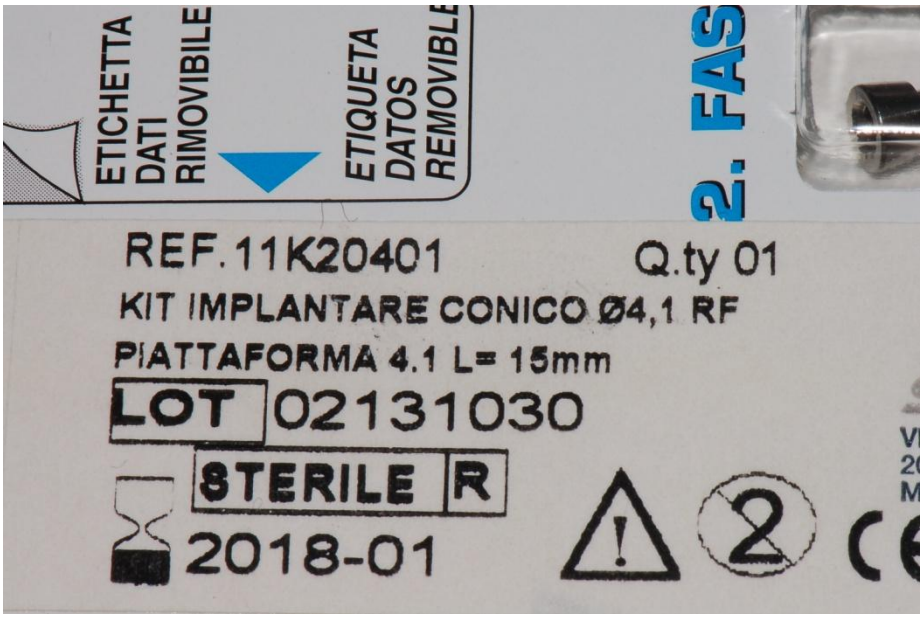



Fig. 2.1.5 A-B: *Tightening with dynamometric ratchet at 25 Ncm*




Group 2 consisted of fixtures (Bone System®, Milano, Italy), 4.1mm in diameter, with internal hexagon connected to the abutment with a sealant (Panavia 21, J.Morita USA Inc, Tustin, California). (Fig. 2.1.6)

Fig. 2.1.6: *Group 2 implants dimensions*



The abutments were connected to the fixtures with a cement through the collar, inserted into the implant using a specific device that ensures the pressure inserting, according to manufacturer's recommendation. (Fig.2.1.7; Fig.2.1.8; Fig.2.1.9A-B)

Fig. 2.1.7: *Implant Group 2*

Fig.2.1.8: *Abutment and collar*

Fig. 2.1.9 A-B: *Collar insertion whit pressure device*

The cement was mixed according to the manufacturer's protocol and applied on the axial surface of the internal portion of the implants to minimize hydrostatic pressure during hardening. Abutments were cemented on the implants with a load of 5 kg maintained for 10 minutes. (Fig.2.1.10A-B; Fig.2.1.11 A-B)

Fig. 2.1.10 A-B: *Abutment cementation*

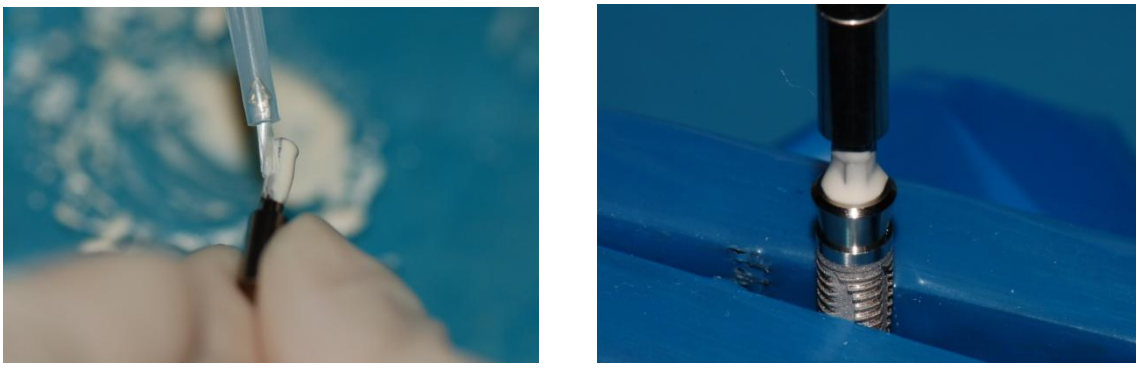


Fig. 2.1.11 A-B: *Abutment cementation and removing excess cement*

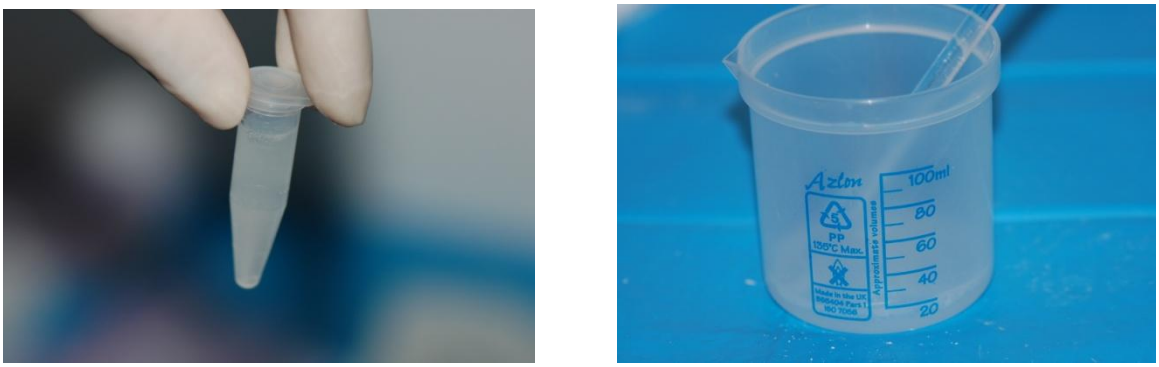
Only one investigator carried out the mixing and cementing procedures at room temperature and also the screwing the connection screw with dynamometric ratchet.

The abutments, previously autoclaved at 121°C for 30 min, were attached to sterile implants, according to the instructions of the manufacturer, under sterile conditions, using sterile instrumentals and gloves.

Saliva Collection

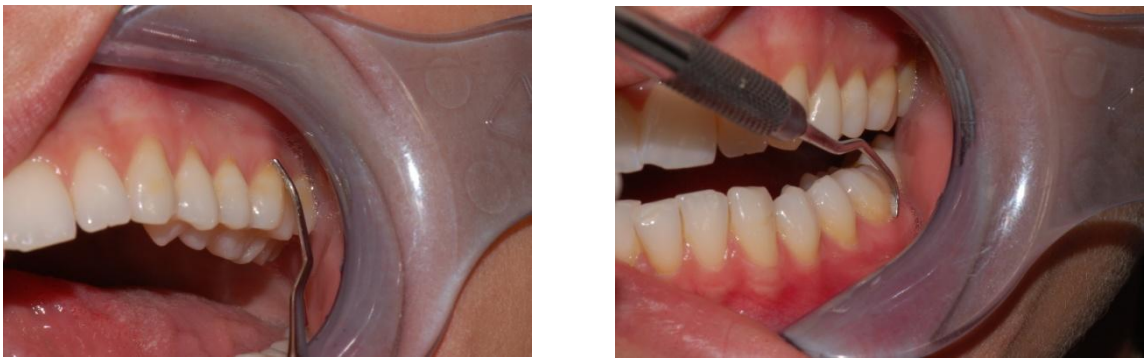
For saliva collection, five healthy patients aged between 31 and 60 years (mean age, 37 years) were enrolled in the study. Two milliliters of non-stimulated saliva were collected from each subject and mixed in final tube. (Fig. 2.1.12 A-B)

Fig. 2.1.12 A-B: *Saliva collection*



In addition, samples of supra-gingival biofilm from the subjects' first maxillary and mandibualr molars were taken with individual curettes and added to the tube.(Fig.2.1.13:A-B)

Fig.2.1.13 A-B: *Sample of supra-gingival biofilm*



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 in the teeth. Patient 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 participation.(Tab. 2.1.1)

Tab. 2.1.1: *Inclusion Criteria*

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

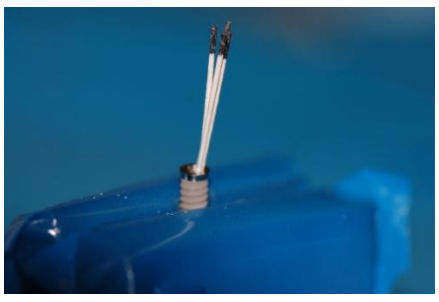
The collected of saliva samples was performed every the morning for 14 days, taking care not modify the customary hygiene.

The study was approved by the local ethics committee, and all experiments were undertaken with the understanding and written consent of each subject and according to ethical principles.

Microbiological assessment

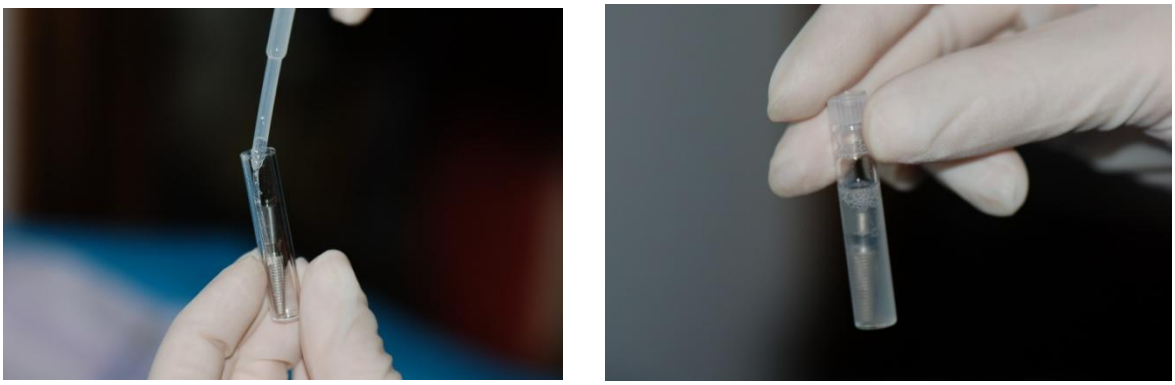
Before the implant-abutment connection, samples from the internal parts of the implants were collected with sterile paper points to be used as negative control for the bacteria contamination. (Fig. 2.1.14)

Fig. 2.1.14 A-B: *Samples collection from the internal part of the implant Group1(A) Group2(B) to be used as negative control*



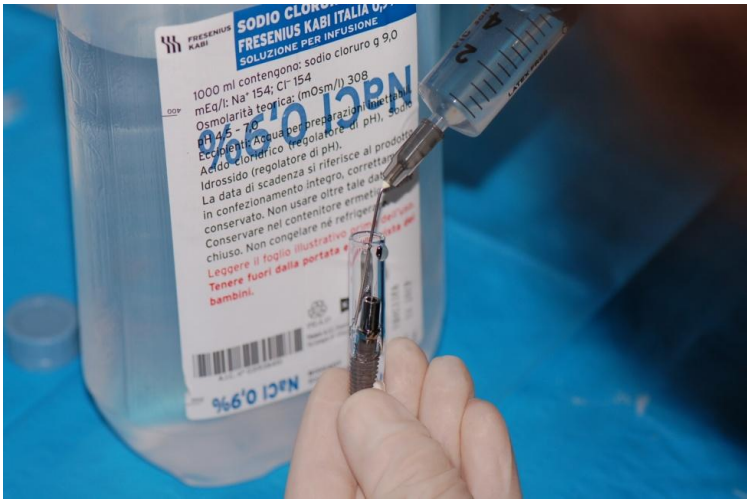
Under aseptic conditions, in a laminar flux hood and using sterile instrumental and gloves, the abutment were attached to implants following the manufacturer's instructions.
Next, assemblies were washed twice with a sterile physiological solution (0.9%NaCl) and dried with sterile gauze pads and then, 4 assemblies each group immersed into micro-tubes containing 200 µl of human saliva. This supernatant volume was sufficient enough to ensure that the micro-gap between implant and abutment (IAI) was totally covered by the saliva.

Fig. 2.1.15 A-B: *Implant immersed in 200 µl of human saliva. The IAI is totally covered.*



1 assembly for group was immersed into micro-tubes containing 200 µl of sterile physiological solution (0.9%NaCl) and was used as negative control. (Fig.2.1.16)

Fig. 2.1. 16: *Implant immersed in 200 µl of sterile physiological solution, used as negative control*



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

Fig. 2.1.17: *Micro-tubes containing samples at 37°C*

Every morning the plaque and saliva into the micro-tubes was partially changed with the new saliva samples.

Fig. 2.1.18: *Change of saliva samples*

After incubation, the assemblies were aseptically disconnected, placed on sterile absorbing paper, washed with NaCl 0.9%, and externally dried with sterile gauzes. (Fig. 2.1.19 A-B, Fig. 2.1.20 A-B, Fig. 2.1.21 A-B, Fig. 2.1.22 A-B)

Fig. 2.1.19: *Samples immersed in saliva after 14 days of 37° C incubation. A Group 1 and B Group 2*

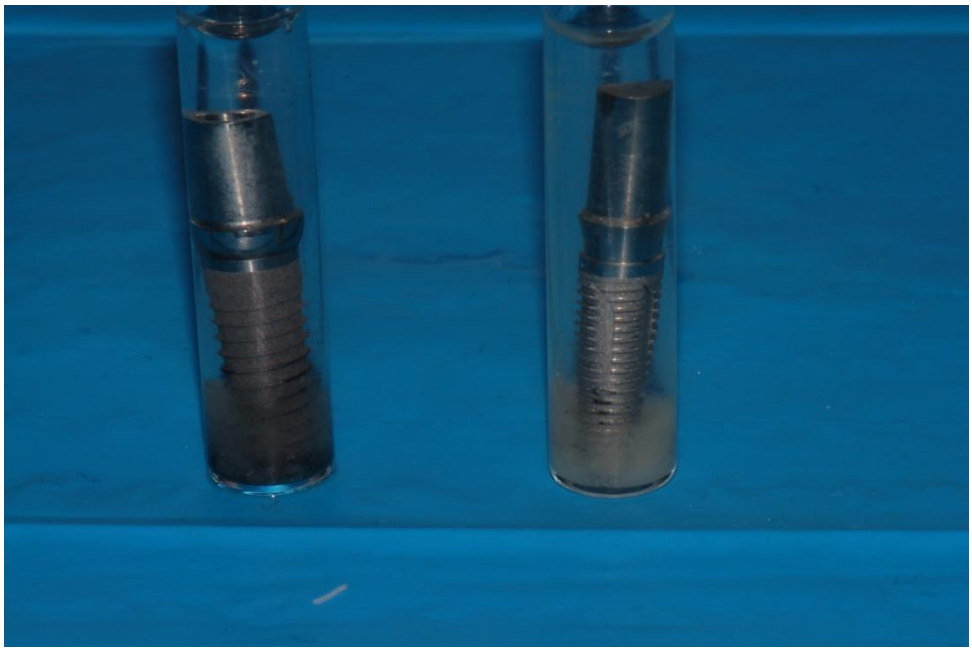


Fig. 2.1.20: *Samples immersed in NaCl 0,9% after 14 days of 37°C incubation. Group 1 and Group 2. Negative control.*

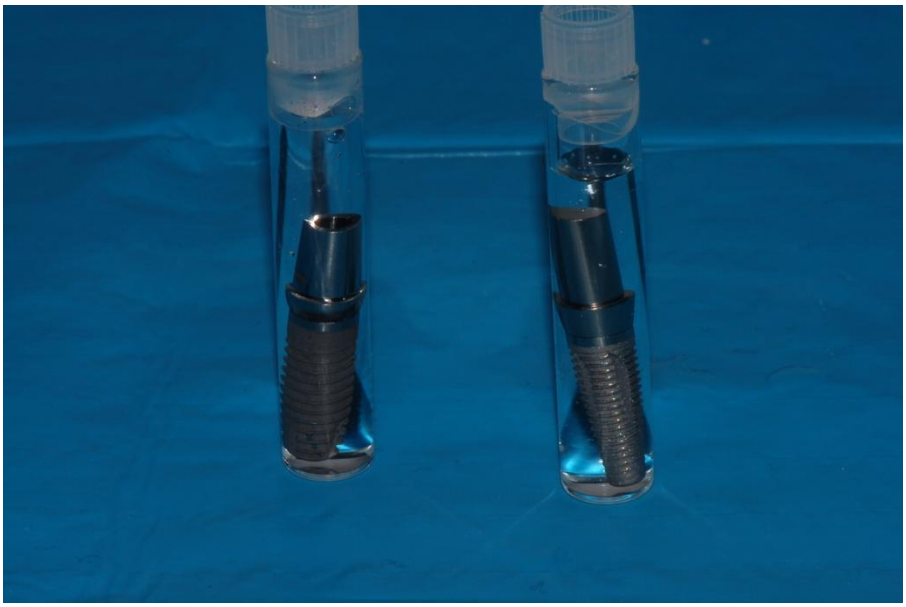


Fig. 2.1.21: *Samples washed with NaCl 0,9%. A Group 1; B Group 2.*

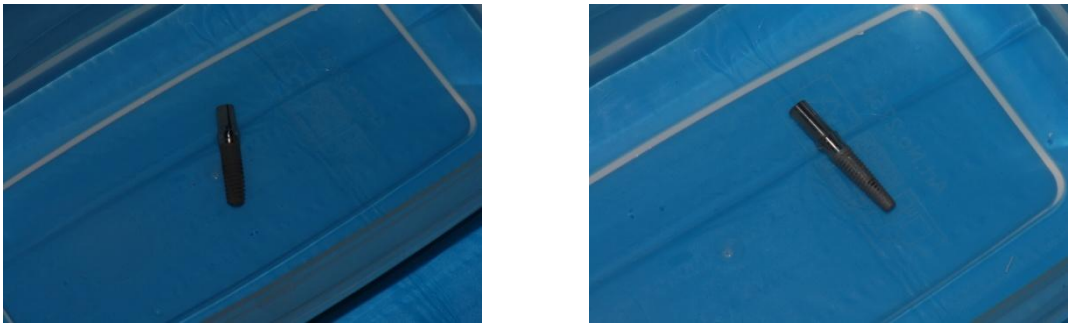
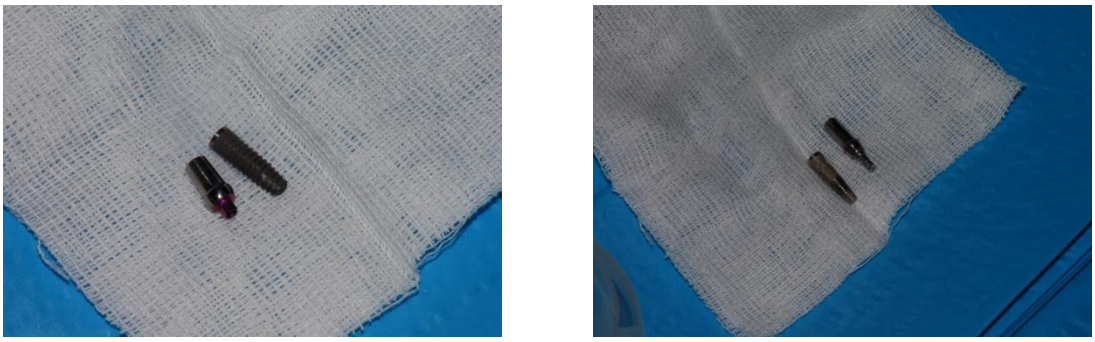
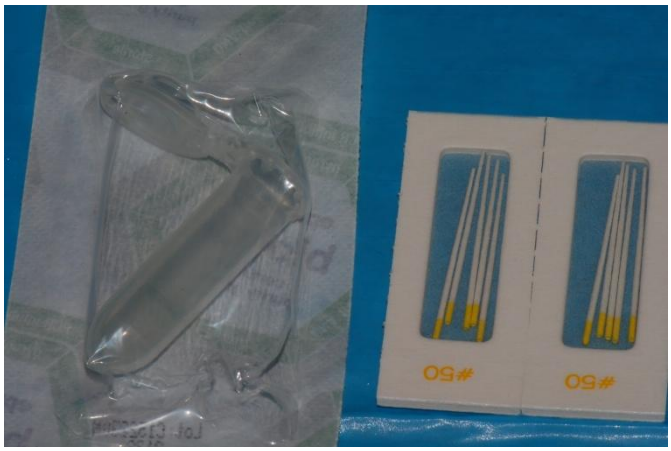


Fig. 2.1.22: *Samples disconnected and dried with sterile gauzes. A Group 1 and B Group 2.*



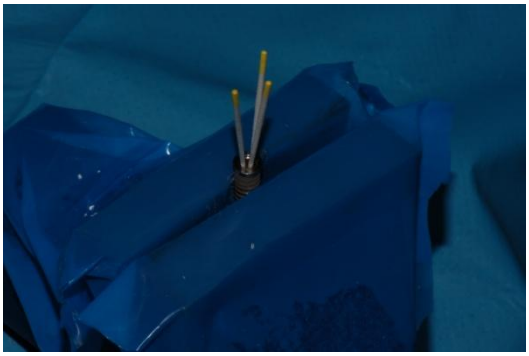
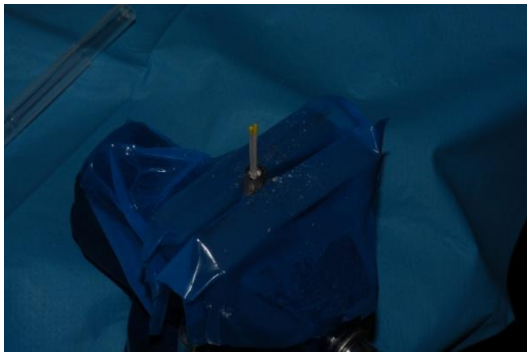
The samples from the internal parts of the implants were collected using a kit, consisting in 10 sterile absorbent paper tips and sterile Eppendorf tubes. (Fig. 2.1.23)

Fig. 2.1.23: *Perio-analysis kit:sterile Eppendorf tube and 10 sterile absorbent paper tips.*



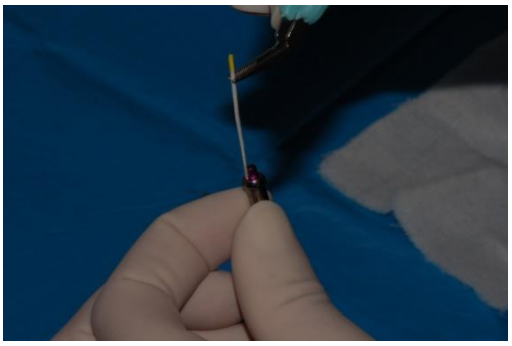
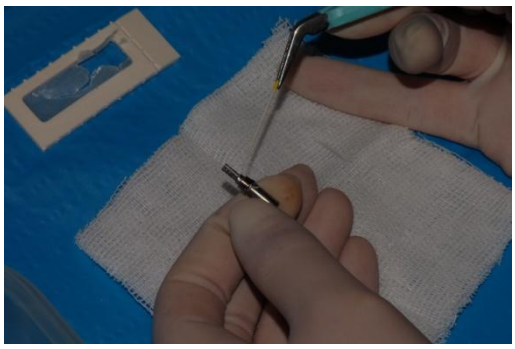
One drop of RNA-and DNA-free water [Water Molecular Biology Reagent (SIGMA) code W4502] was placed inside the implant connection and three paper tips were insert for 30s. (Fig. 2.1.24 A-B)

Fig. 2.1.24 A-B: *Paper tips insert for 30s inside the internal part of the implants. A Group 1 and B Group 2.*

The connection surface of the abutment was wetted with a drop of RNA-and DNA-free water and smeared with two paper tips. (Fig. 2.1.25 A-B)

Fig. 2.1.25 A-B: *Bacteria samples collected on the abutments surface. A Group 1 and B Group 2.*

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) in the provided mailing envelopes.

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, 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) end manufactured by Metabion GmbH (Martinsried, Germany). The bacterial primers used are derived from previously published ribosomal 16S sequences [44,45] and have been adapted to the real-time PCR conditions. (Fig. 2.1.26; 2.1.27; 2.1.28;2.1.29)

Fig. 2.1.26: *safety cabinet for paper point preparation and bacterial lysis before DNA extraction*

Assays were carried out on the RotorGene® Q thermal cycling system (Qiagen,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, Germany).

Fig. 2.1.27: *Qiagen Robot for automated DNA extraction with silicon column*

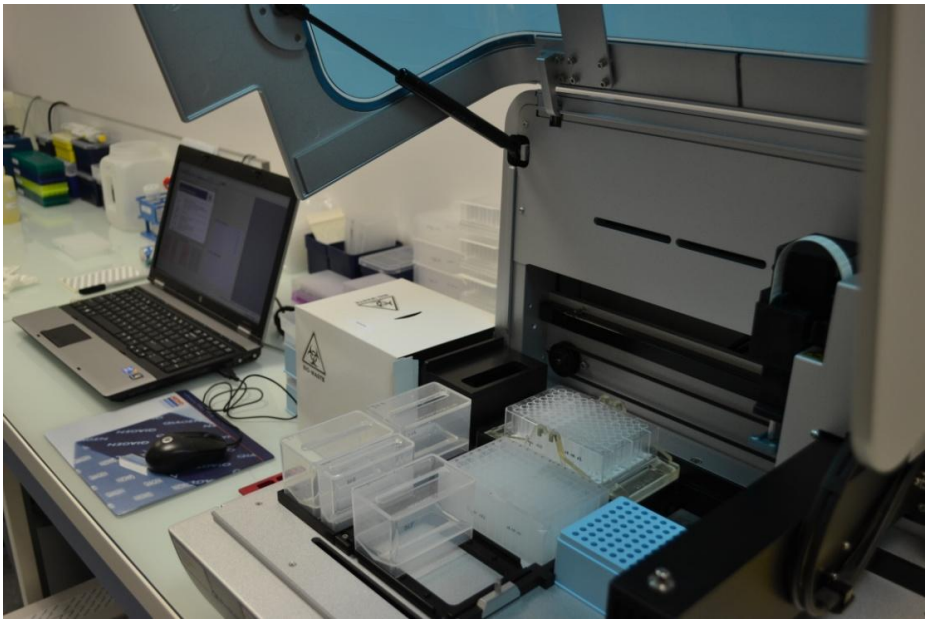


Fig. 2.1.28: *the Room and robot Qiagility use for primer mix preparation and distribution*

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).

Fig. 2.1.29: *RotorGene thermocycler*

2.2 Effects of different implant-abutment connections on micro-motions and stress distribution: prediction of micro-gap formation

Aim of the study

The aim of this study was to analyze micro-movements and stress distribution at the implant-abutment interface in two types of implant-abutment connection systems.

Study design

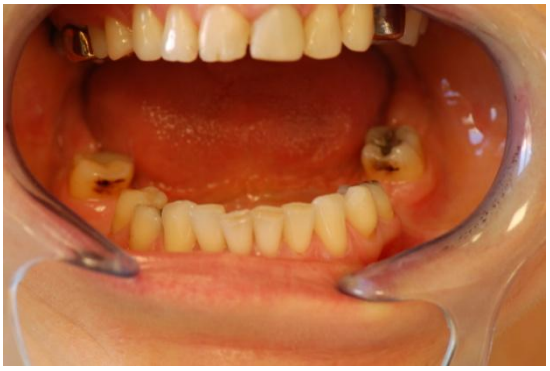
For this study a computer model was used, but based on a real model. An impression of the patient's jaw was used for determinate the real implant position.

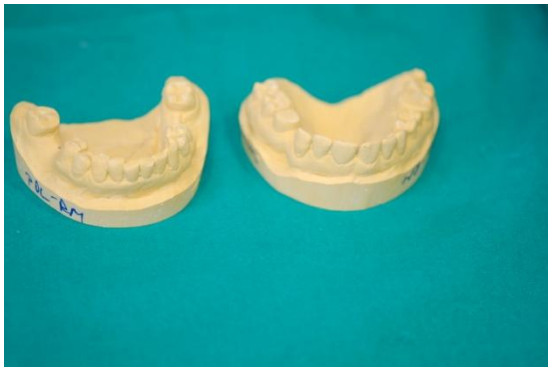
An implant-abutment three-dimensional model design for each types of implant system was implemented. The different patterns of micro-movements and stress distribution under a simulated real occlusal loading for 5 years was analyzed with Finite Element Analyze (FEA).

Real Model

A patient that needed a implant-prosthetic rehabilitation was selected. (Fig. 2.2.1 A-B)

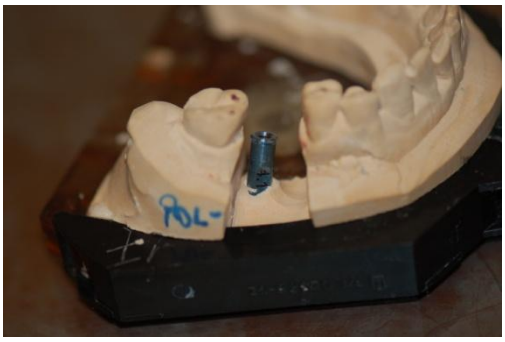
Fig. 2.2.1 A-B: *Clinical exam and study models*





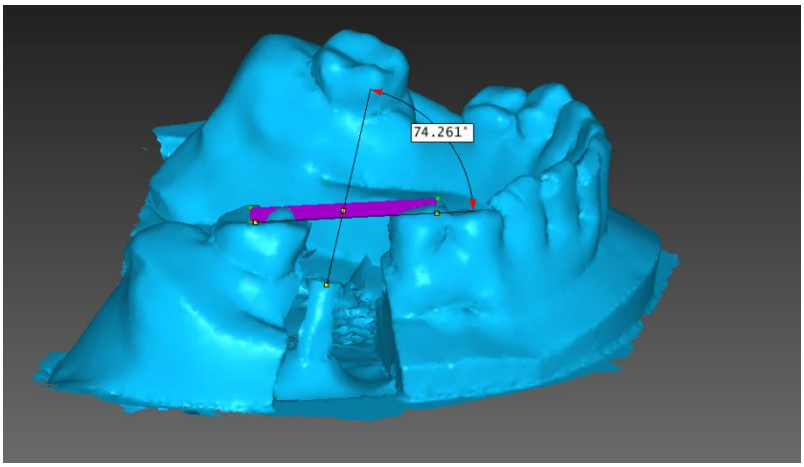
The implant was inserted following two surgical standard procedures. At the moment of prosthetic rehabilitation was performed a precise impression of the implant position, of the antagonist jaw and an diagnostic wax-up. (Fig.2.2.2 A-B)

Fig. 2.2.2 A-B: *Implant position and direction*



All this devices were scanned thanks to a laser-scanner 3D (Nikon Metrology, MMDx100, Nikon Corporation, Chiyoda, Tokyo, Japan) and saved on a 3D software (Nikon Focus, Nikon Corporation, Chiyoda, Tokyo, Japan). (Fig. 2.2.3)

Fig. 2.2.3 A-B: *Laser-scanner 3D and 3D model*



Three dimensional model design

The titanium implant, the abutment, the abutment screw, the titanium collar, and the superstructure of each implant system were designed using three-dimensional (3D) Computer Aided Design (CAD) software (Autodesk Inventor, Autodesk, San Rafael, CA, USA).

Measurements of all components were made from product sample.

The dimensions of the implants were chosen to the real dimensions used for to rehabilitate the patient: 4.5 mm in diameter and 11 mm in length for the implant with screwed implant-abutment connection and straight abutment with diameter 4.5 mm; 4.1 mm in diameter and 10 mm in length for the implant with cemented implant-abutment connection, straight abutment with base diameter 3.8 mm and collar diameter 3.7-4.6 and length 2 mm. (Fig. 2.2.4, Fig. 2.2.5)

Fig. 2.2.4: *Implants design dimensions. Group 1.*

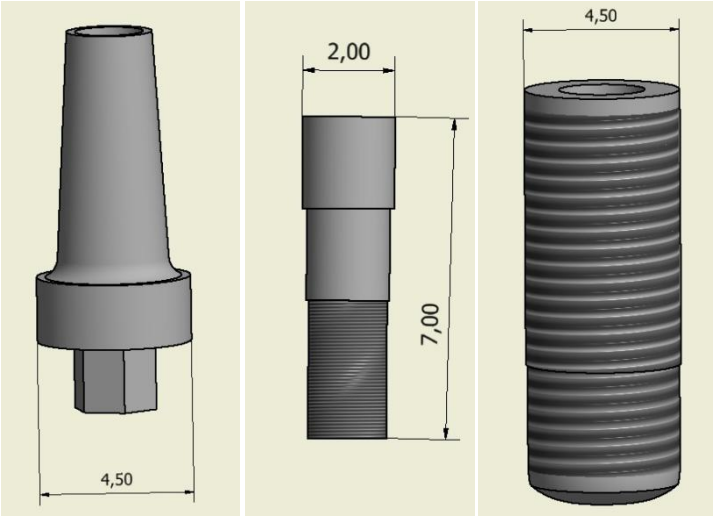
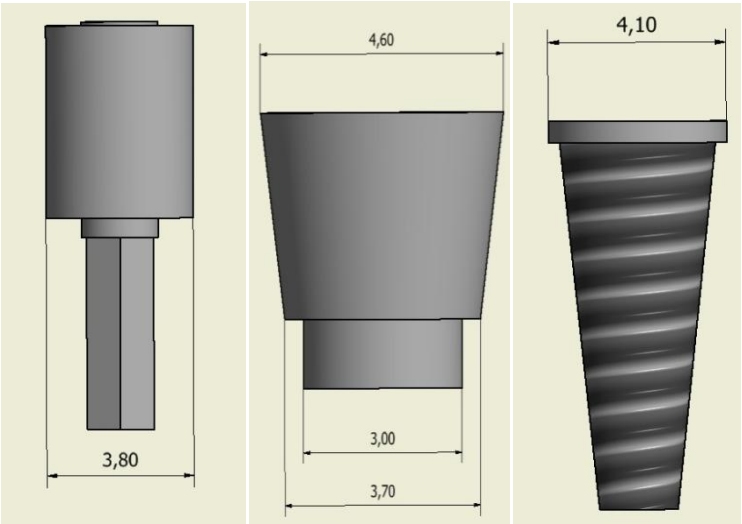


Fig. 2.2.5: *Implant design dimensions. Group 2.*



To simulate a fixed prosthesis, simplified superstructures modeled as columns were overlapped over the abutment, in both models.

The basic shapes of the finite element implant model were illustrated using computer-aided design (CAD). The CAD software used for visualization of the design and manufacturing processes was Autodesk Inventor (Autodesk, San Rafael, CA, USA), which enabled the visualization of precise three-dimensional models.(Fig. 2.2.6; Fig. 2.2.7)

Fig. 2.2.6: *3D implant model Group 1.*

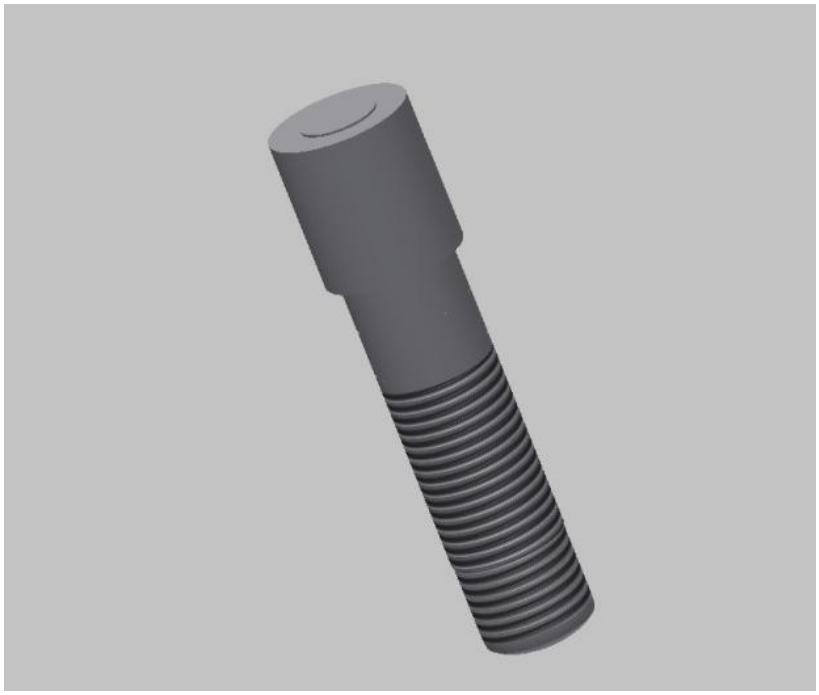
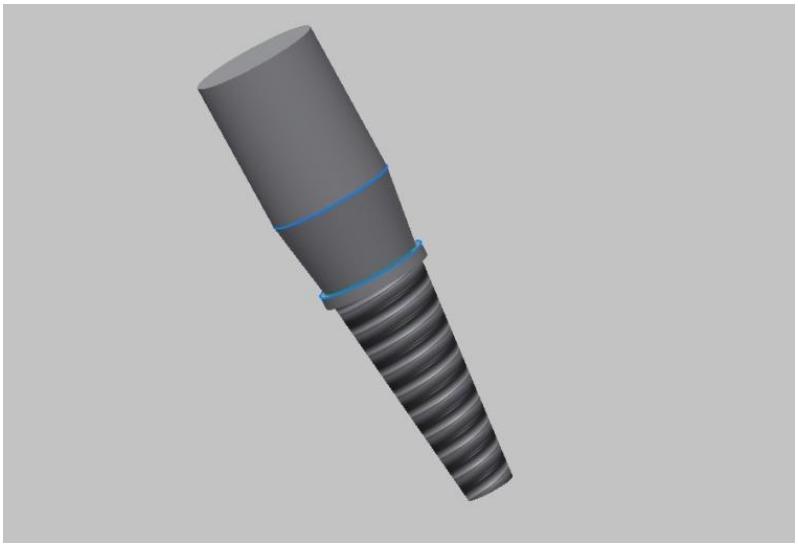


Fig. 2.2.7: *3D implant model Group 2.*



The same software was used to FEM calculations.

The CAD files were saved in IPT Format and were mashed. The CPU of the personal computer used a speed of 2,6 GHz.

Views of two FEM models are show in Figure 2.2.7. The analysis was performed using three-dimensional finite element analysis with tetrahedral solid elements. The screw-joint type model (Fig. 2.2.8 A) was composed of 165578 nodes and 105301 elements, and the cemented-joint type model (Fig. 2.2.8 B) was composed of 174195 nodes and 109856 elements. The material properties used for the FEM analysis are shown in Table 2.2.I

Fig. 2.2.8 A-B: *View of two types of finite element model. A) Screw-joint type model B) cemented-joint type model*

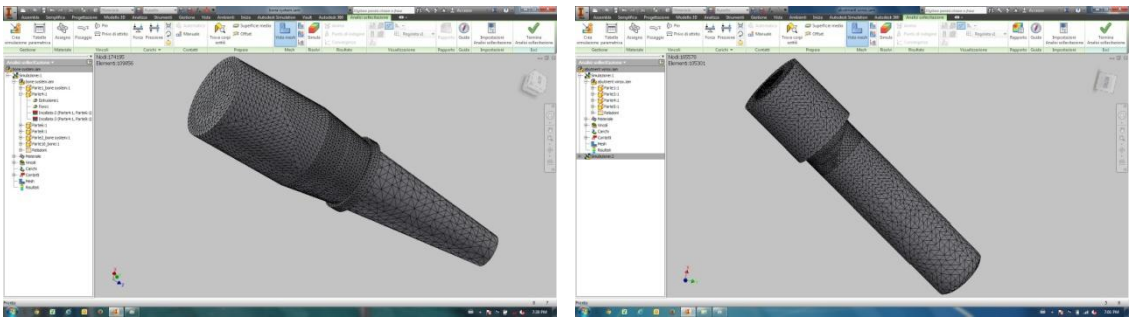


Table 2.2.I. *Material properties used in Finite Element Analysis study of both models.*

	Young Modulus (MPa)	Poisson's Ratio (-)
Pure Titanium	110,000	0.35
Titanium alloy	110,000	0.35
Posterior mandible (Type II)	5,500	0.33
Cortical Bone	13,700	0.33
Crown	68,900	0.28
Cement	10,000	0.25

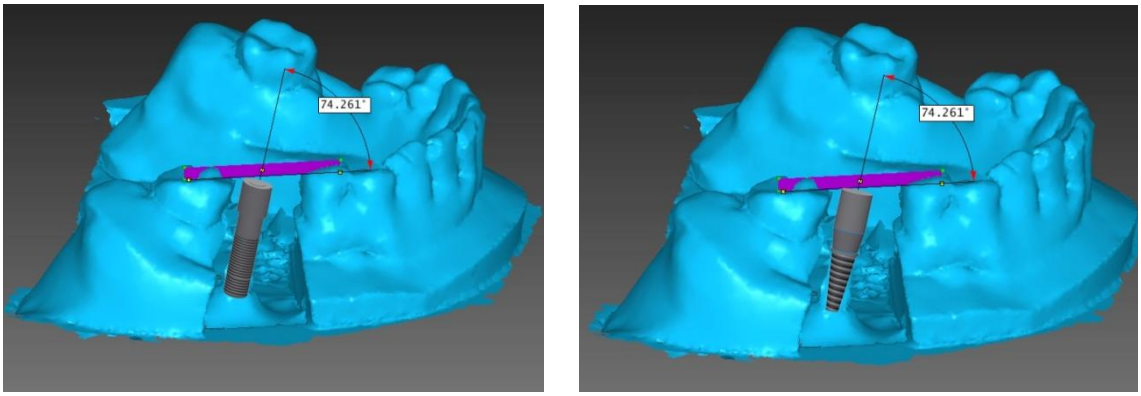
All 3D models were considered to be homogenous, linear and isotropic.

In addition, the interface between the superstructure and abutment was assumed continuous.

Both models assumed that the implant was embedded in the lower right molar region, in the plaster model's position, like the real model.(Fig. 2.2.9 A-B)

The implant assemblies were positioned and fixed at 74,2° to the ideal occlusal plane like determinate by the real model.

Fig.2.2.9 A-B: *Real model implants positions. A Group 1; B Group 2.*



In this study, complete osseointegration between the implant and the surrounding bone was simulated, and the model was constrained in the X-,Y-, and Z-directions on implant surface.

Additionally, the contact element was used for contacting surfaces between the abutment, abutment screw, collar, and implant because nonlinear analysis in consideration of the contact. Furthermore, the assumed coefficient of friction was set to 0.5 between all components as titanium alloy and pure titanium.[27]

To tighten the abutment screw, the screw joint-type model was set with a preload of 380 N (manufacturer's recommended tightening torque 25 N-cm). (Fig. 2.2.11; Fig.2.2.12)

Fig. 2.2.11: *Tightening screw simulation. Group 1.*

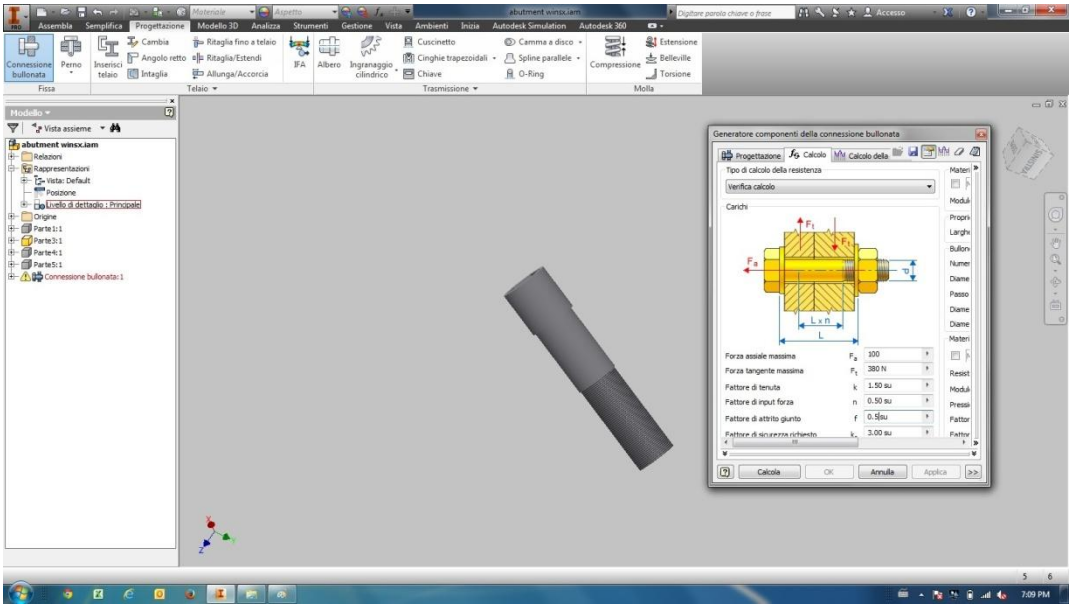
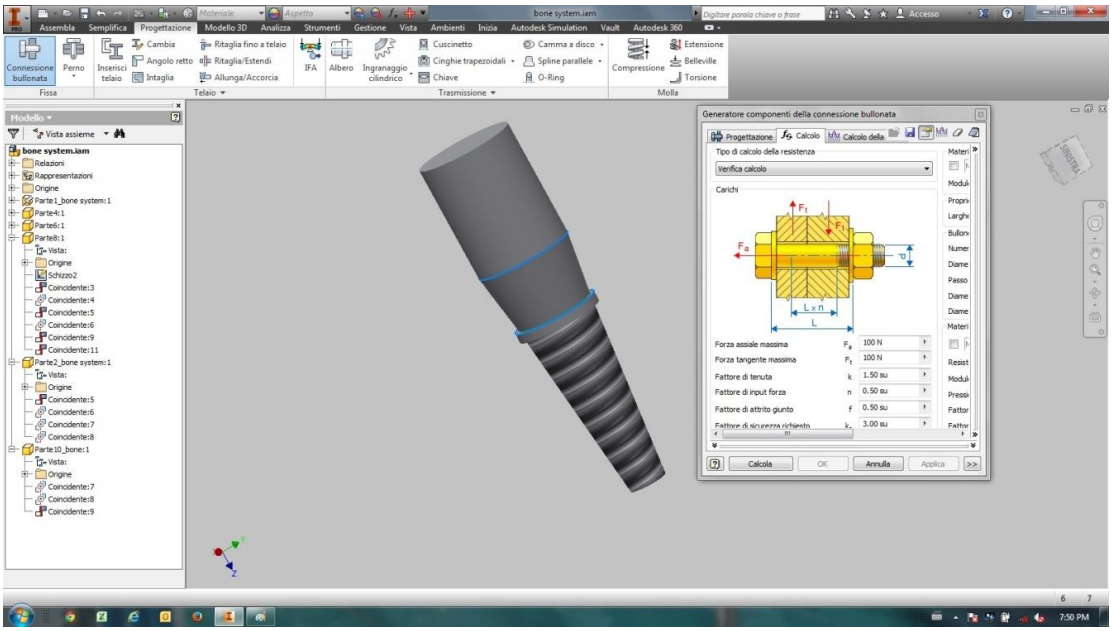
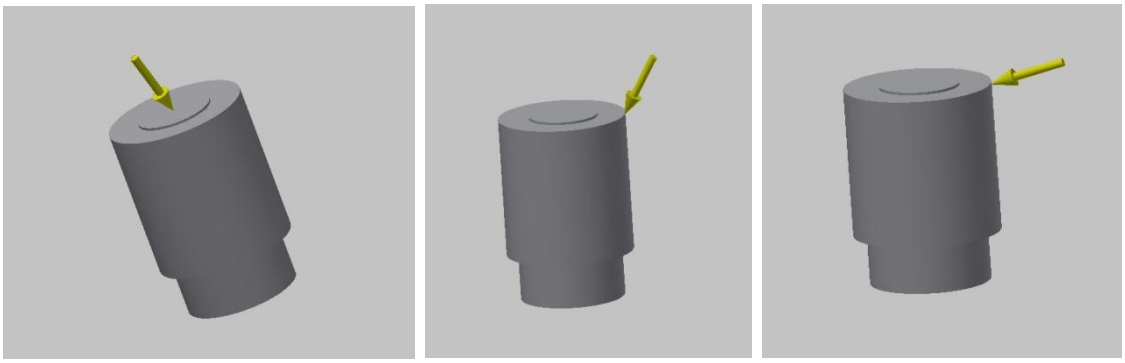


Fig. 2.2.12: *Implant-collar-abutment assembly simulation. Group 2.*



Linear dynamic analysis by FEM was used to calculate the transient response of the dental implant system. Thus, load was applied at the occlusal surface of the dental implants, as shown in Figure 2.2.13, because occlusal forces are complex in vector.

Fig. 2.2.13: *Directions of load applied on occlusal surface*



In addiction was performed a fatigue analysis. From FEA analysis, an average stress of 100 MPa to the connection was generated and it was used to perform a fatigue analysis. The specifics of analysis are reported below.

Analysis options

The following analysis options were chosen

- The - Signed VonMises - analysis method was used
- Analysis was performed on the highest 100 percent of stresses
- Stresses below 0N/mm2 were not considered in the fatigue calculations
- Nodal stress averaging was performed. This may increase the predicted Life

All Parts were used in the analysis

Material

A Steel material was analyzed.
MPa Stress units selected

Material = Material
Modulus of Elasticity = 10300
Ultimate Tensile Strength = 434
Endurance Limit = 100
Number of cycles at Endurance = 100

Method

The Stress Based analysis method was chosen.
Stress based analysis is a direct solution and is generally quicker than Strain based analysis.

Advantages

- Quicker to perform than Strain based methods.
- Disadvantages
- Not applicable for low cycle analysis (less than 10000 cycles to failure)
- Pessimistic. May predict failure when no failure occurs in reality.
- High cycle fatigue only (Greater than 10000 cycles)
- More input may be required

Modifiers

Stress Concentration Factor Kf =1
Reliability = 1
Miscellaneous = 1
Surface Finish = 1.000

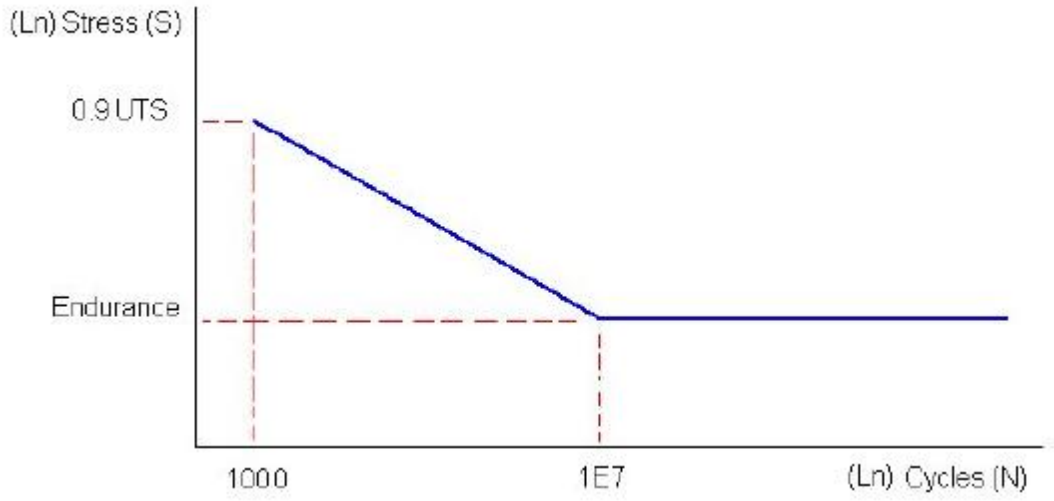
Overall factor (Kf) = 1.000

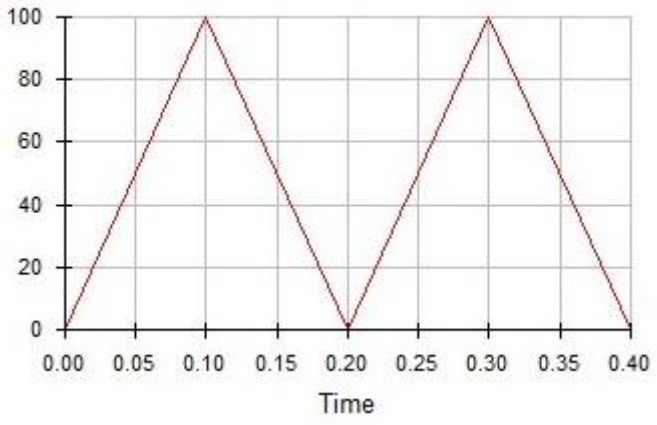
Loading

There are 1 load cases in the Linear Static model.
Only the Load Cases/Curves defined below were used in the fatigue analysis.
The loading history defined, acts as a multiplier to your Linear Static Stress results.

The stress was applied every 0.1 second and was removed every 0.1 second for 1,000,000 cycles, corresponding to ca 5 years of chewing, as show in Fig. 2.2.14.

Fig. 2.2.14: *Fatigue analysis conditions*





2.3 Evaluation of peri-implant bacterial microflora in implants systems with different connections types. An *in vivo* study.

Aim of the study

The aim of this *in vivo* study was to evaluate the peri-implant bacterial microflora and any clinical changes in the two different types of implant-abutment connections.

Study design

For this study were selected 20 patients, 10 male and 10 female, aged between 27 and 62 years (mean age 46 year) that needed to implant-prosthetic rehabilitations in premolar or molar area.(Tab. 2.3.I)

Tab 2.3.I: *Study design..*

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

The patients were selected following a precise protocol:

- ✓ Presence of single or partial edentulous and need to dental implant treatment
- ✓ Age >18 years
- ✓ No presence of systemic diseases (*Molbelli 2006*)
- ✓ No use of drugs, like antibiotics, anti-inflammatory and corticosteroids, at least 3 months
- ✓ Absence of acute oral diseases not treated (caries, endodontic lesions, periodontal disease)
- ✓ Absence of bleeding on gentle probing (<0.25N), PPD <5mm and absence of radiographic bone loss assessed in paralleled periapical radiographs (*Lang & Berglundh 2011)*
- ✓ No surgical treatment for peri-implantitis or periodontal disease at least 6 months
- ✓ Absence of parafunction or disfunction
- ✓ No smokers (at present or during the 12 months prior to the study)
- ✓ No pregnant or lactating patients

All patients had accepted and signed an informed consent. This study was approved by ethics committee.

The patients had showed throughout the course of the study a good compliance and an adequate control of oral hygiene (O'Leray's Plaque Index 20%).

Implant-Prosthetic treatment

Before the implant treatment each patient was subjected to the following steps:

- ✓ Alginate impression for diagnostic study model
- ✓ Tc Dentascan
- ✓ Diagnostic wax-up
- ✓ Surgical template
- ✓ Treatment of acute oral diseases
- ✓ Supragingival debridement and instructions for a good oral hygiene

The surgical treatment for to insert dental implants followed a precise protocol:

- ✓ Local anesthesia with adrenalin 1:100.00

- ✓ Rinse with clorexidina 0,2% for 60 seconds
- ✓ 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..)

Were inserted a total of 50 implants: 25 with screwed implant-abutment connection (WinSix, BioSAF IN, Ancona, Italy) Group 1; 25, with cemented implant-abutment connection (Bone System, Milan, Italy) Group 2.

All the implants were rehabilitated 3 or 4 months after surgical treatment. Were performed total thickness flaps for to discover the implants and were used healing screws for 14 days. Subsequently, the implants were rehabilitated using metal-ceramic crowns.

Also for the prosthetic, was followed a precise protocol, different between cemented and screwed abutment.

For the implant system with a screw that connected fixture and abutment, Control Group, was inserted the abutment into the implant and jointed with connection screw using first the specific screwdriver and then the dynamometric ratchet, tightening the screw to 30 Ncm like indicated by manufacturer.

For the implant system with abutment cemented into the implant, Experimental Group, thanks to specific device was inserted the cone Morse collar inside the fixture. The abutment, then, was cemented with Panavia 2.1 (J.Morita, USA, Inc, Tustin, California). The cement was mixed according to the 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 minutes. Excess cement was removed with a scaler. Only one investigator performed mixing and cementing procedures at room temperature and the same investigator carried out tightening of the screws.

After screwed or cemented implant abutment connection, crowns or bridges were cemented on the implant.

After cementation of the crowns or bridges, the excess cement was removed with a scaler. The patients were subjected to professional hygiene every three months and after at least 360 days from the prosthetic rehabilitation was performed the collection of peri-implant bacteria samples. The follow-up was between 360 days and 2 years with a mean follow-up of 484 days, approximately 1 years and 4 months. (Tab. 2.3.II)

Tab 2.3.II: *Study design..The red sites are the implants chosen for this study.*

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

In this study were included only 20 implants, one implant for each patient, chosen in causal modalities, preferring the first molar.

Were chosen, so, three upper right first molars, six upper left first molars, two lower left first molars and nine lower right first molars.

The implants were divided into two group: Group 1 implant with screwed implant-abutment connection; Group 2 implant with cemented implant-abutment connection.

Microbiological assessment

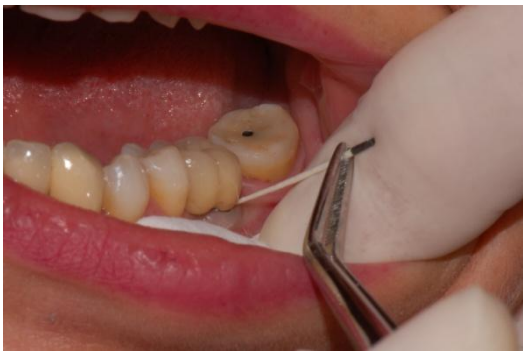
A total of 50 implants were inserted, 25 with screwed abutments and 25 with cemented abutments. No postoperative complications or loss of implant occurred.

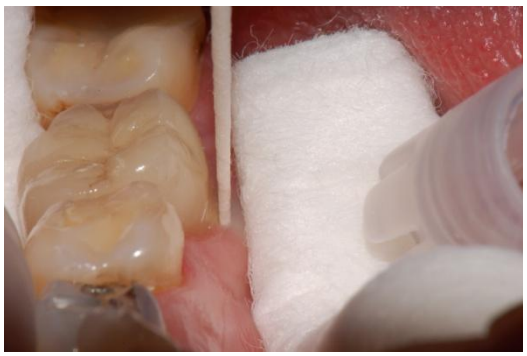
At least 360 days, 12 months, after the prosthesis, was performed the bacteria collection. Only 20 implants were selected for this study, one for each patient, chosen randomly.

Sampling for microbiological analysis from all groups was performed by a single researcher. 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 seconds. (Fig. 2.3.1)

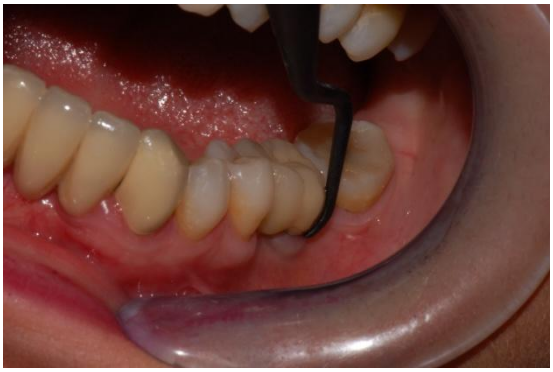
Fig. 2.3.1 A-B: *Absorbent paper tip inserted into the peri-implant sulcus.*

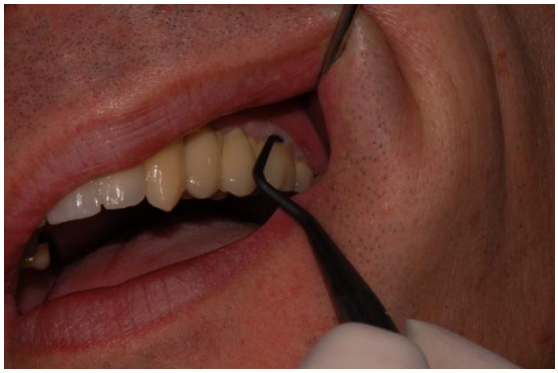




Prior to subgingival plaque sampling, supragingival plaque was removed from implants and neighboring teeth using a Teflon curettes (Implacare, HuFreiedy, Chicago IL; USA), without penetrating the gingival or pei-implant sulcus. (Fig. 2.3.2 A-B)

Fig. 2.3.2: *Removal of supra-gingival plaque using a Teflon curettes.*



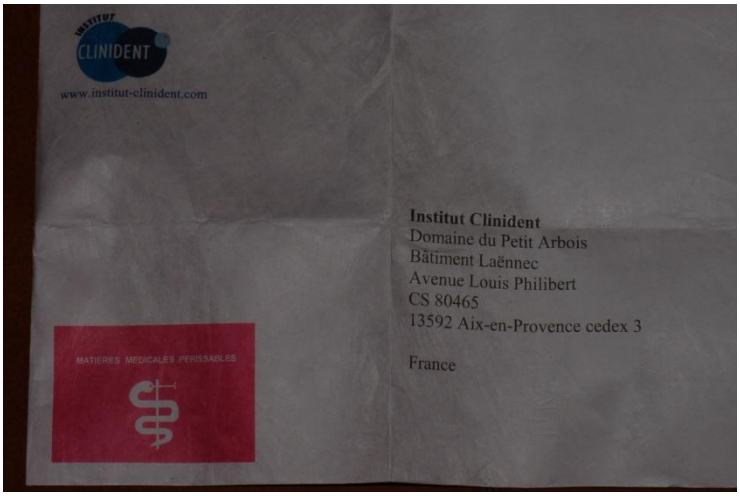


Cotton rolls were used for relative isolation and sampling sites were dried with an air pistol.

The paper tips, with the samples, were placed into the Eppendorf tubes and were sent for microbiological analysis to the laboratory Institut Clinident SAS (Aix en Provence, France) in the provided mailing envelopes. (Fig.2.3.3, Fig.2.3.4)

Fig. 2.3.3: *Eppendorf tube containing paper tips*

Fig. 2.3.4: *Send to Institute Clinident SAS, Aix en Provence for microbiological analysis*

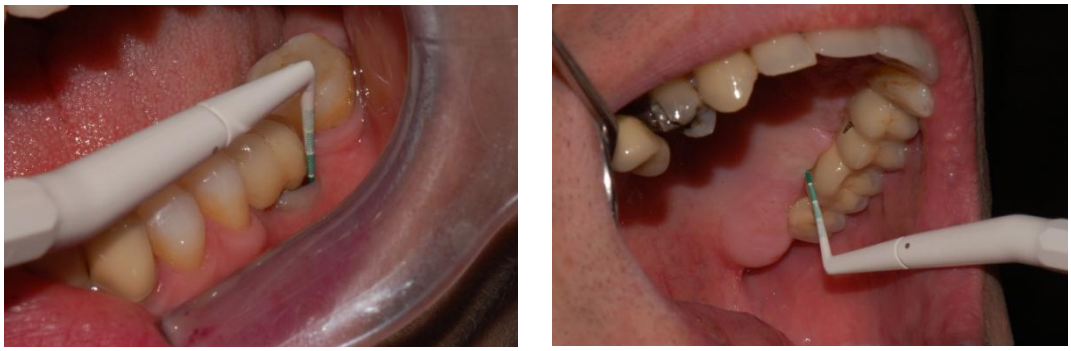


After the sampling, was performed, also, a clinical exam, in 6 sites around implant using a millimetric probe HAWE CLICK-PROBE (Hawe Neos Dental, Bioggio, Suisse) and were recorded 5 parameters: (Fig. 2.3.5 A-B)

- PPD (Probing Pocket Depth)
- m SBI (Modified Sulcus Bleeding Index) *(Mombelli et al. 1987)*
- m GI (Modified Gingival Index) *(Mombelli et al. 1987)*
- m PI (Modified Plaque Index) *(Mombelli et al. 1987)*
- REC (Gingival Recession)

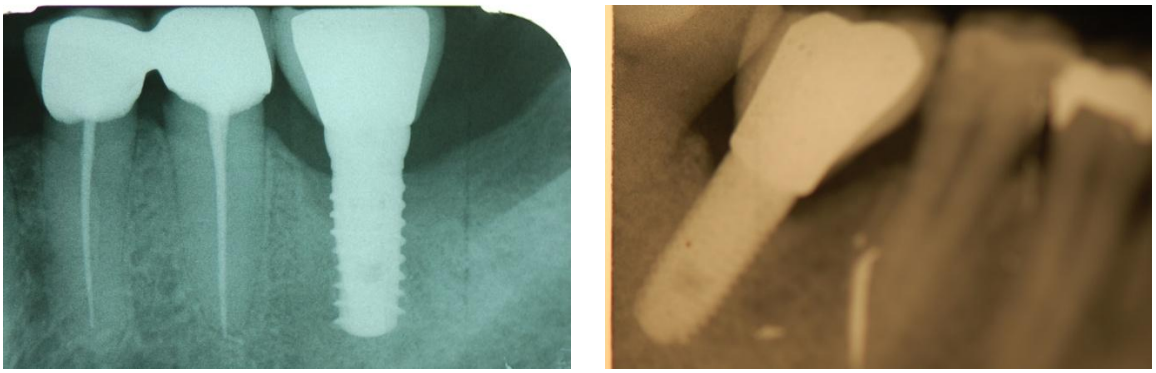
m SBI, m GI, m PI were evaluated in 4 sites around implant and were calculated the mean value getting the implant's score. For the REC was evaluated the presence or absence.

Fig. 2.3.5 A-B: *Probing around implants and recording the periodontal parameters*



Also, was carried out an endoral peri-apical radiograph. (Fig. 2.3.6)

Fig. 2.3.6 A-B: *Periapical radiographs*



At the end of study all collection data were inserted in a work sheet (Microsoft Excel) and analyzed by a specific statistic software (SPSS V10).

Quantitative real-time PCR assays

Quantitative real-time PCR was carried out for Total Bacterial Count (TBC) and for 9 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).*

Quantitative real-time PCR assays were performed in a volume of 10 1l composed of 1 9 QuantiFast® SYBR® Green PCR (Qiagen, 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) end manufactured by Metabion GmbH (Martinsried, Germany). The bacterial primers used are derived from previously published ribosomal 16S sequences [44,45] and have been adapted to the real-time PCR conditions.

Assays were carried out on the RotorGene® Q thermal cycling system (Qiagen,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, 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).

3. RESULTS

3.2 Bacterial leakage of saliva in Implants with different implant-abutment connections: an *in vitro* study

In this study were tested a total of 10 implants, 5 for each group. The implants were assembled and inserted in tubes containing human saliva and were put in incubator a 37°C for 14 days.

After 14 days, changing the saliva every morning, the implant-abutment were disassembled and the bacteria samples were performed using sterile paper points inserted into the implant for 30 seconds. The samples were sent to Istitut Clinident s.a.s. (Aix en Provence) and analyzed by real-time PCR.

PCR methodology provided Genomic DNA extraction from sample and test for micro-organisms 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 an high permeability to bacteria for the Group 1, with screwed implant-abutment connection, as showed in Table 3.1.I and 3.1.II.

After 14 days, bacterial contamination was observed in a total of 5 assemblies of 8, 4 assemblies for Group 1, with screwed connection, and only 1 assembly for Group 2, with cemented connection. The contamination, then, was 100% for the Group 1 implants and 25% for the Group 2 implants, but it was not statistically significant.(P=.074, P<.05; Fisher-test)

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 the Group 1 versus 7,2E+04 (±14,4E+04) for the Group 2, with a high level of significance (P=.0001, P<.05; T-test)

For the Group 1, with screwed connection, the bacterial contamination was positive for all tested bacteria except *Aggregatibacter actinomycetemcomitans* and *Candida albicans,* but all bacteria were below the threshold of pathogenicity. (Fig. 3.1.1)

Tab 3.1.I: *Group 1, screwed implant-abutment connection, average results.*

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			

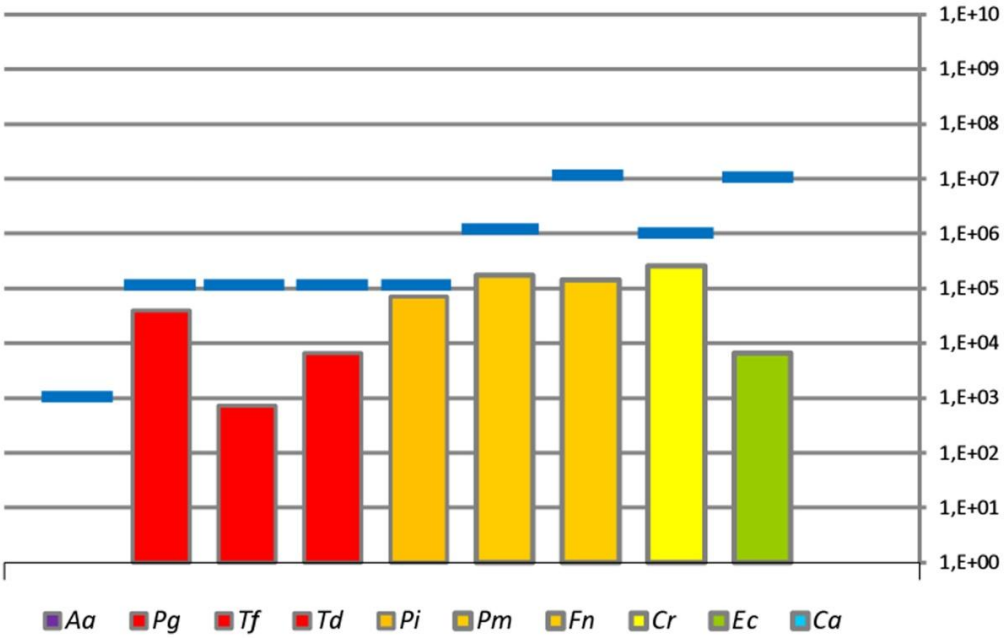
Pathogenic load:** the amount of detected bacteria in sample; ****Pathogenic threshold:** Represents a specific microbiological pathogenic load above which antibiotic therapy is recommended in order to reduce risk of tooth or implant attachment loss (periodontal disease or peri-implantitis). *****Status:** 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.

Tab.3.1.II: *Group 2, cemented implant-abutment connection, real-time PCR*

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			

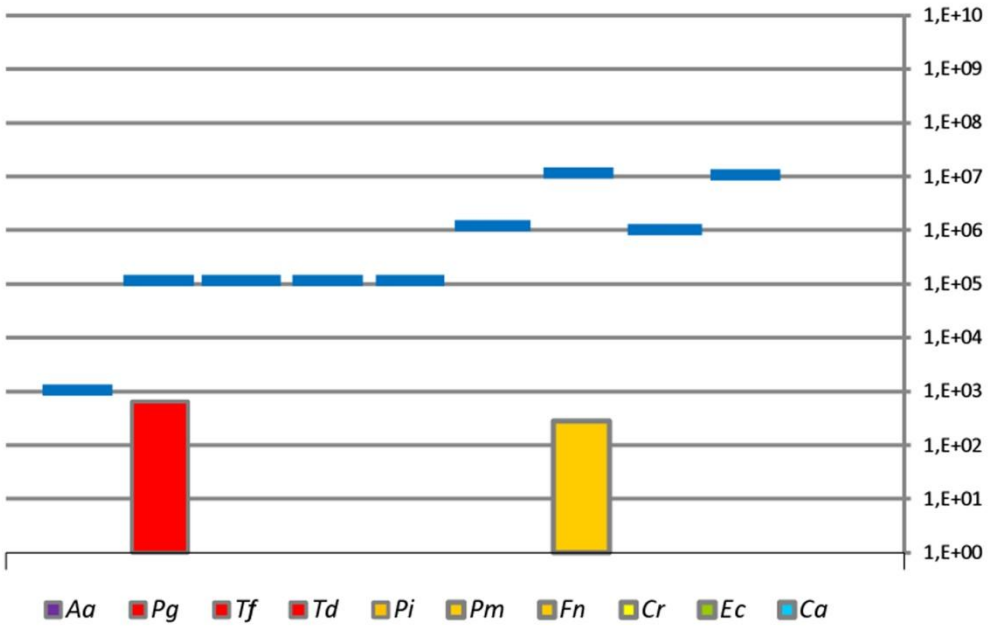
Pathogenic load:** the amount of detected bacteria in sample; ****Pathogenic threshold:** Represents a specific microbiological pathogenic load above which antibiotic therapy is recommended in order to reduce risk of tooth or implant attachment loss (periodontal disease or peri-implantitis). *****Status:** 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.

Fig. 3.1.1: *Group 1 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.*

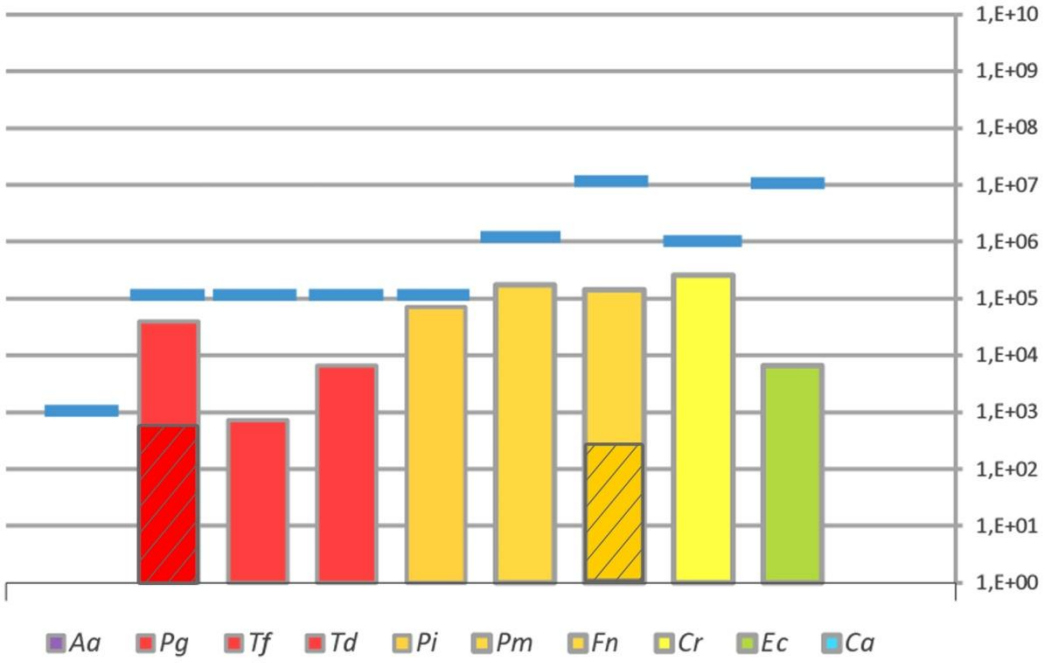
Fig.3.1.2: *Group 2 bacterial leakage, average results*



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.*

For the Group 2, with cemented connection, the bacterial contamination was positive for only two bacteria *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, and well below the threshold of pathogenic.

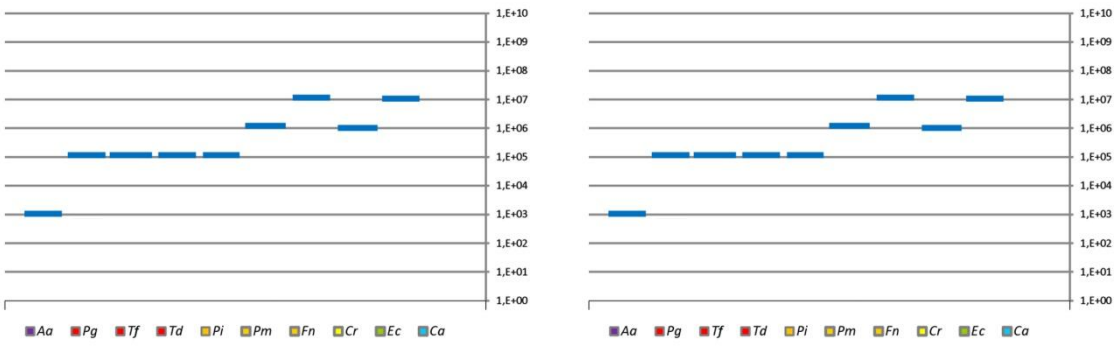
Fig. 3.1.3: *Bacterial colonization Group 1 and Group 2. Graph of comparison.*



Overlapping rectangles of the histogram. Dashed rectangles represent results of Group 2.

All the samples used as negative controls and collected from implants before contamination testing showed no colonization for evaluated species. (Fig. 3.1.4 A-B)

Fig. 3.1.4 A-B: *Results of samples used as negative control.*



3.2 Effects of different implant-abutment connections on micro-motions and stress distribution: prediction of micro-gap formation

In this study were evaluated the micro-motion and stress distribution to the connections, by FEA and fatigue analysis.

An impulsive load of 100 N was applied at the occlusal surface and was 30° from the occlusal plane. The application points of the load were in the center of simulated crown with a disto-mesial direction, on simulated crown edge with a mesio-distal direction and lateral with a mesio-distal direction, because the masticatory forces are complex in vector.

In addiction was performed a fatigue analysis. From FEA analysis, an average stress of 100 MPa to connections was generated and it was used to perform a fatigue analysis. The stress was applied every 0.1 second and was removed every 0.1 second for 1,000,000 cycles, corresponding to ca 5 years of chewing.

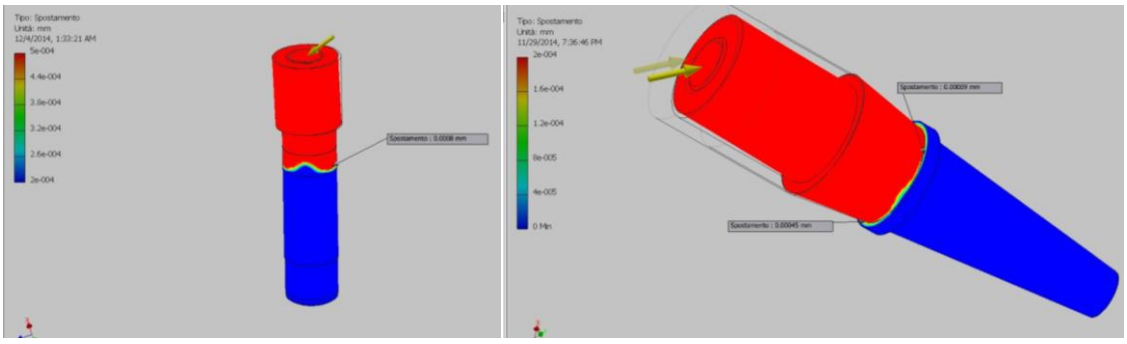
The implant assemblies were positioned and fixed at 74,2° to the ideal occlusal plane like determinate by the real model.

The results showed significant micro-motion occlusally at the implant-abutment connection, and gradually decreased towards the inferior region of the abutment.

The higher micro-motion was evaluated when the force was applied laterally to simulated crowns both for the screwed connection and for the cemented connection.

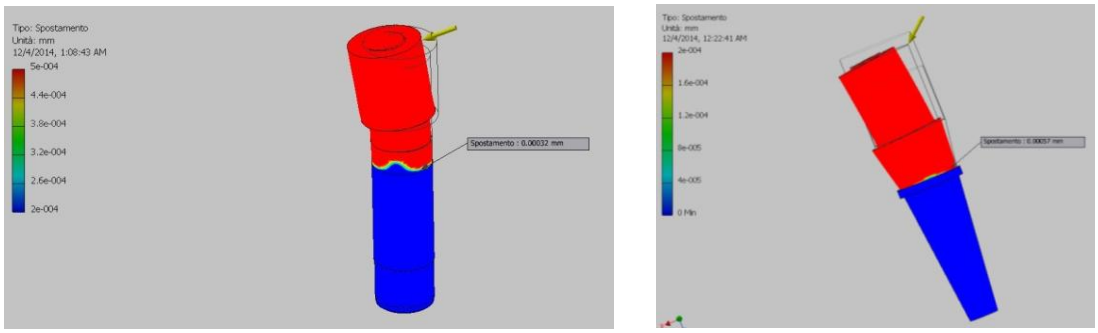
There were no significant differences between the two groups as shown in Fig. 3.2.1A-B, 3.2.2A-B, 3.2.3 A-B. At the level of system connection, instead, the micro-motions were higher in the Group 2, 1,5 µm, than in the Group1, 0,225 µm, when the force was applied on edge of simulated crown.

Fig. 3.2.1 A-B: *micro-motions when the force is applied on the simulated crowns center with a 30° inclination. Group 1, screwed connection, and Group 2, cemented connection.*



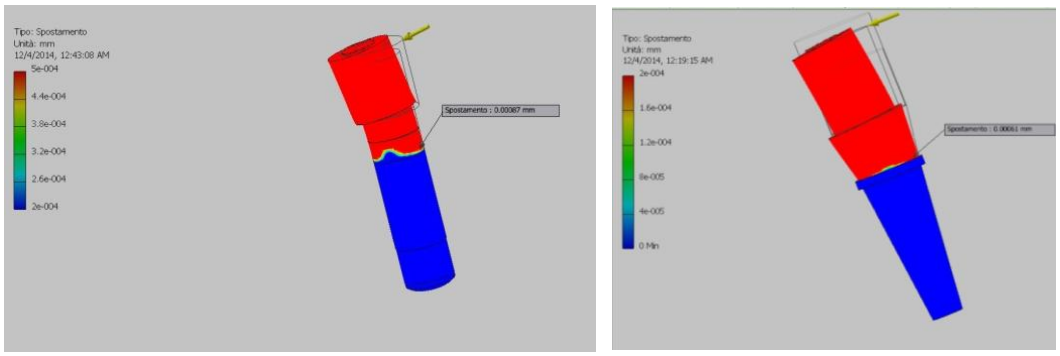
The micro-motions were 0,8 µm for the Group 1 and 0,09-0,45 µm for the Group 2.

Fig. 3.2.2A-B: *micro-motions when the force is applied on the simulated crowns edge with a 30° inclination. Group 1, screwed connection, and Group 2, cemented connection.*



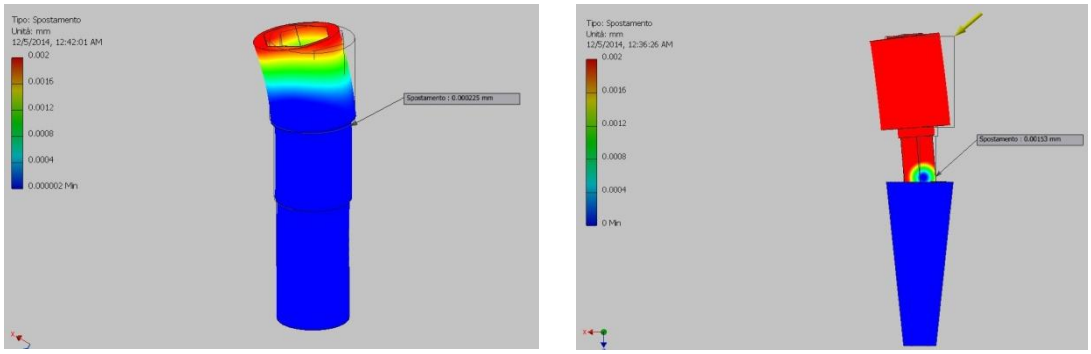
The micro-motions were 0,32 µm for the Group 1 and 0,57 µm for the Group 2.

Fig.3.2.3A-B: *micro-motions when the force is applied laterally to the simulated crowns. Group 1, screwed connection, and Group 2, cemented connection.*



The micro-motions were 0,87 µm for the Group 1 and 0,51 µm for the Group 2.

Fig. 3.2.4A-B: *micro-motions of the internal screw for the Group 1 and the internal portion of abutment for Group 2.*

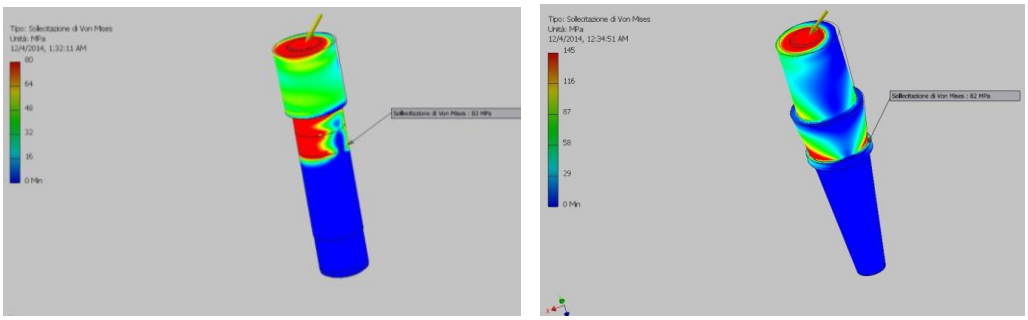


The micro-motions were 0,225 µm for the Group 1 and 1,5 µm for the Group 2.

Also the stress was estimated to the level of implant-abutment interface, and it was similar for the two implant-abutment connection. No significant differences were found as show in

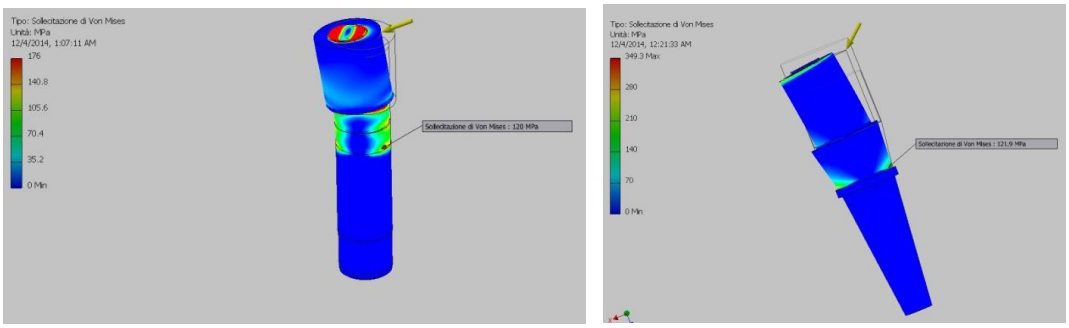
fig. 3.2.5 A-B, 3.2.6 A-B, 3.2.7A-B. The system connection, instead, showed a higher stress in the Group 1, 36,87 Mpa, then in the Group 2, 0 MPa, as shown in fig. 3.2.8A-B.

Fig. 3.2.5A-B: *stress at IAI's level when the force is applied at the center of simulated crowns with 30° inclinations. Group 1, screwed connection, and Group 2, cemented connection.*



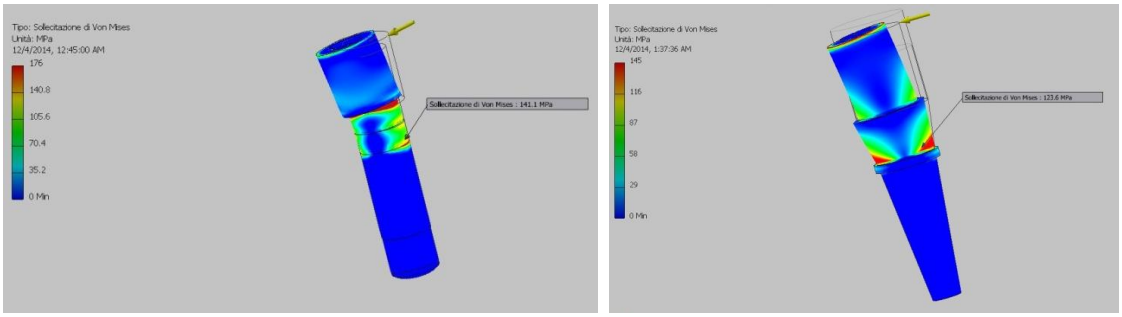
The stress was 83 MPa for the Group 1 and 82 MPa for the Group 2.

Fig. 3.2.6A-B: *stress at IAI's level when the force is applied at the edge of simulated crowns with 30° inclinations. Group 1, screwed connection, and Group 2, cemented connection.*



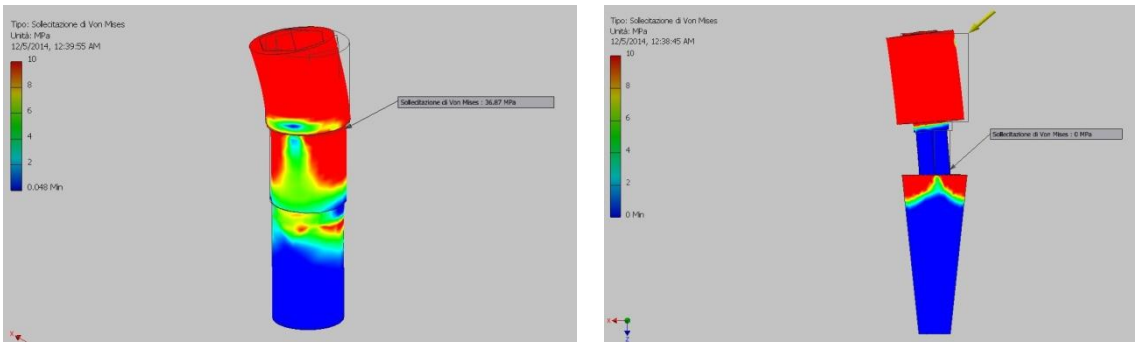
The stress was 120 MPa for the Group 1 and 121,9 MPa for the Group 2.

Fig. 3.2.7A-B: *stress at IAI's level when the force is applied laterally to simulated crowns. Group 1, screwed connection, and Group 2, cemented connection.*



The stress was 141,1 MPa for the Group 1 and 123,6 MPa for the Group 2.

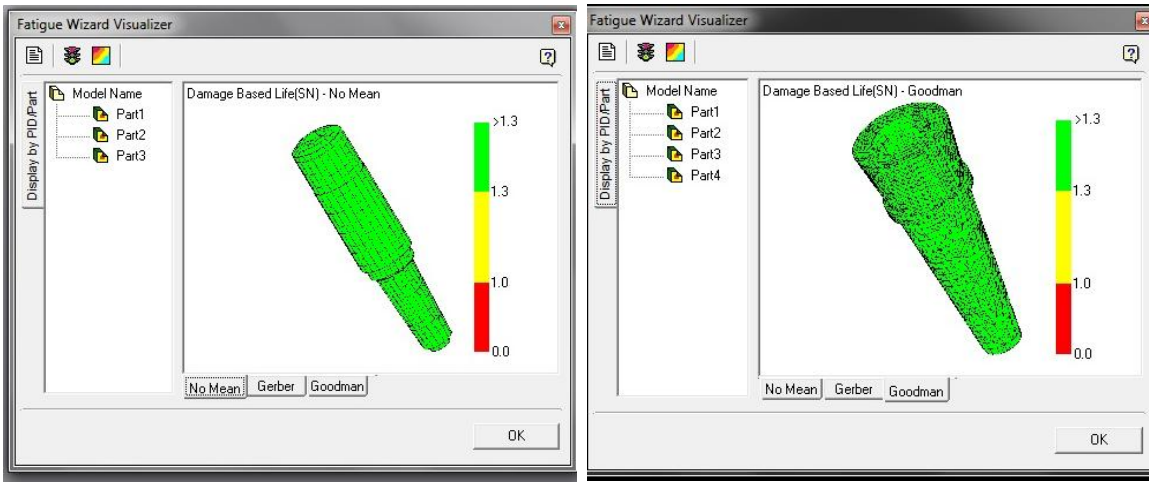
Fig. 3.2.8A-B: *stress of the internal screw for the Group 1 and the internal portion of abutment for Group 2.*



The stress was 36,87 MPa for the Group 1 and 0 MPa for the Group 2.

Taking as parameter the average stress, ca 100 MPa, was performed also the fatigue analysis for 1E+07 cycles. The results showed the same behavior for both implant-abutment connections. The break was hypothesized after more than 1E+32 cycles.

Fig.3.2.9A-B: *Results of fatigue analysis. Group 1 and Group 2.*



Results

Desired number of cycles before failure = 1E+07
Predicted Number of cycles to failure = 1E+32

Results

Desired number of cycles before failure = 1E+07
Predicted Number of cycles to failure = 1E+32

3.3 Evaluation of peri-implant bacterial micro-flora in implants systems with different connections types. An *in vivo* study.

In this study were included 20 implants, inserted in 20 patients, 10 with screwed implant-abutment connections and 10 with cemented implant-abutment connections.

Were chosen three upper right first molars, six upper left first molars, two lower left first molars and nine lower right first molars.

Were analyzed the peri-implant micro-flora, after at least 360 days from the prosthetic rehabilitation, using paper points inserted in the peri-implant sulcus for 30 seconds.

The samples were sent to Istitut Clinident s.a.s (Aix en Provence, France) for the real-time PCR analysis.

Was evaluated the presence of 9 bacteria periodontal-pathogens and the Candida albicans.

All the peri-implant sulci analyzed, showed the colonization by bacteria but for the Group 1, with screw-retained abutment, the presence of bacteria was higher than the Group 2, with cement-retained abutment, as showed in Table 3.3.I and 3.3.II.(Fig. 3.3.3)

The average of total bacterial count was 3,7E+08(±1,19) for the Group 1, with screwed connection, versus 2,1E+08 (±0,16) for the Group 2, with cemented connection, without significant differences.(P=.32; P<.05, T-test)

The Group 1 implants showed a micro-flora composed by all the bacteria analyzed, excluding *Aggregatibacter actinomvcetemcomitans* and *Candida albicans.* The bacterial colonization of peri-implant sulci is over the pathogenic threshold for 5 bacteria: *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Prevotella intermedia, Campylobacter rectus*; indicating an high risk of peri-implantitis. (Fig. 3.3.1)

Also the Group 2 implants showed a micro-flora composed by all bacteria analyzed, excluding *Aggregatibacter actinomycetemcomitans*. In this case, the bacterial colonization of peri-implant sulci is over the pathogenic threshold for only 1 bacterium: *Prevotella intermedia*; indicating a low risk of peri-implantitis.(Fig.3.3.2)

A patient presented Candida albicans disease and its presence was found in the analysis.

Tab. 3.3.I: *Group 1, screwed implant-abutment connection, average results*

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			

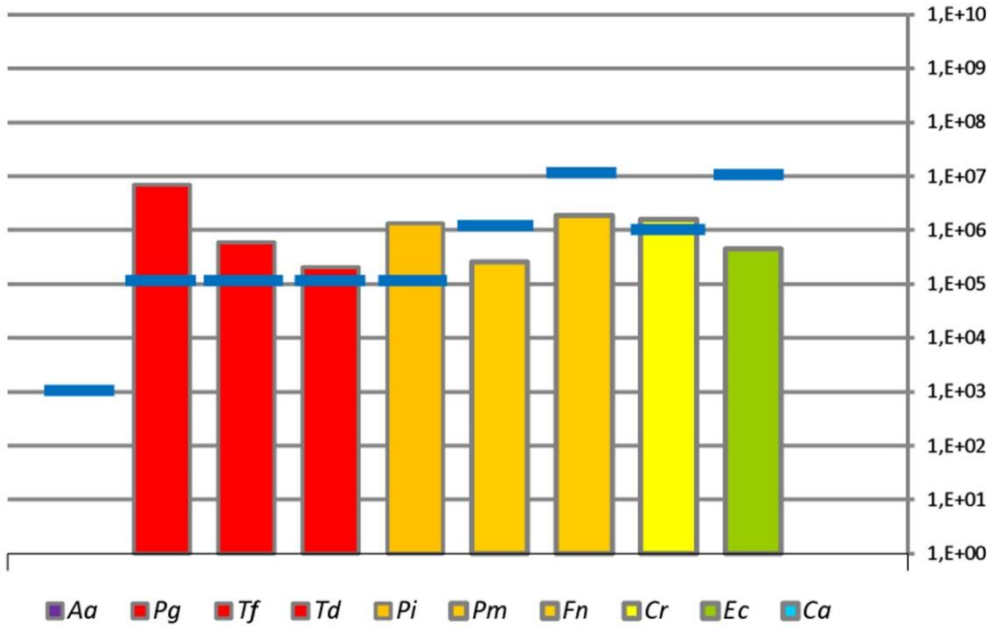
Pathogenic load:** the amount of detected bacteria in sample; ****Pathogenic threshold:** Represents a specific microbiological pathogenic load above which antibiotic therapy is recommended in order to reduce risk of tooth or implant attachment loss (periodontal disease or peri-implantitis). *****Status:** 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.

Tab. 3.3.II: *Group 2, cemented implant-abutment connection, average results*

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			

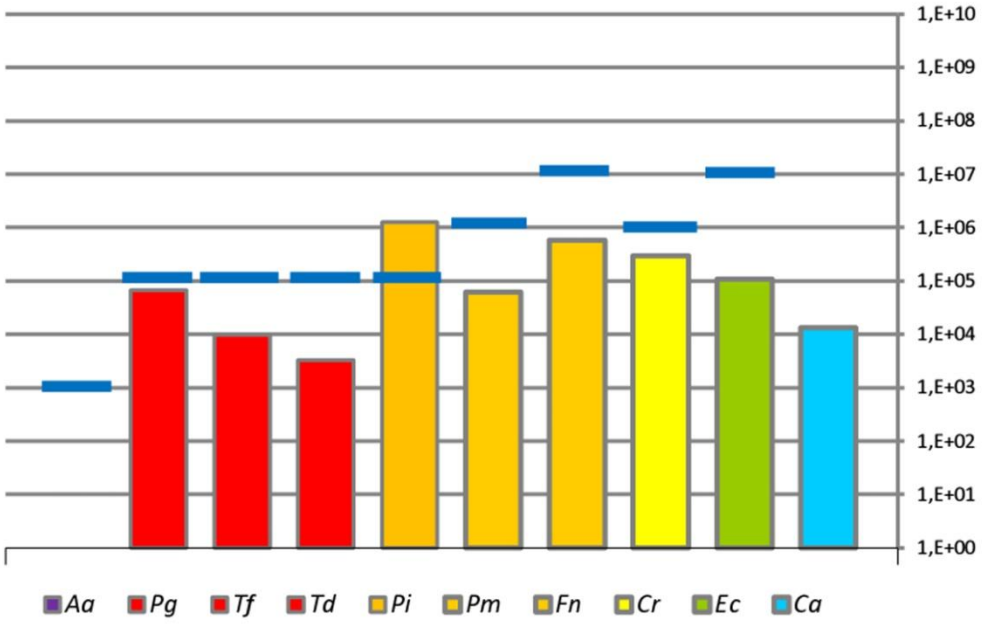
Pathogenic load:** the amount of detected bacteria in sample; ****Pathogenic threshold:** Represents a specific microbiological pathogenic load above which antibiotic therapy is recommended in order to reduce risk of tooth or implant attachment loss (periodontal disease or peri-implantitis). *****Status:** 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.

Fig. 3.3.1: *Group 1bacterial leakage, average results*



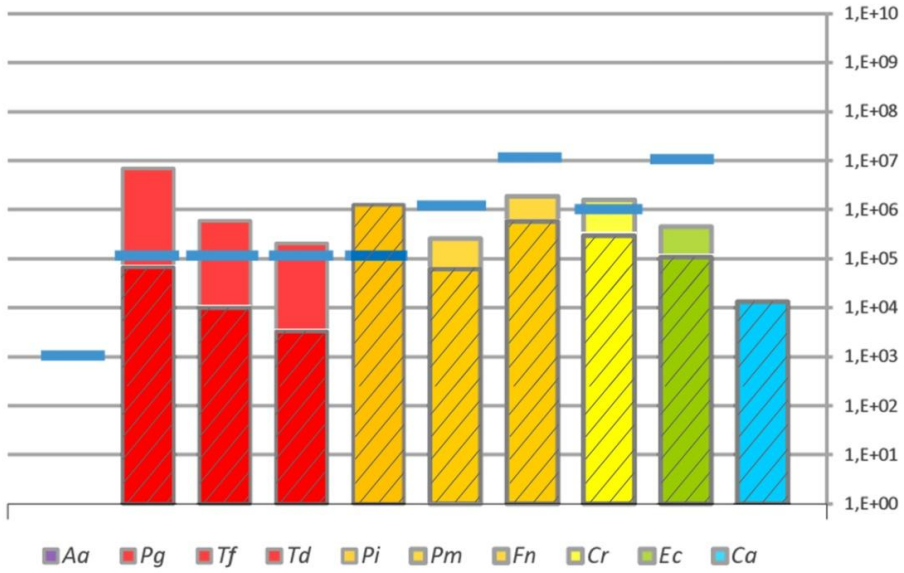
Aa: *Aggregatibacter actinomycetencomitans;* Pg: *Porphyromonas gingivalis;* Tf: *Tannerella forsythia;* Td: *Treponema denticola;* Pi: *Prevotella intermedia;* Pm: *Parvimonas micra;* Fn: *Fusobacterium nucleatum;* Cr: *Campylobacter rectus;* Ec: *Eikenella corrodens;* Ca: *Candida albicans.*

Fig. 3.3.2: *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.*

Fig. 3.3.3: *Bacterial colonization Group 1 and Group 2. Graph of comparison.*



Overlapping rectangles of the histogram. Dashed rectangles represent results of Group 2.

Also the clinical exam showed different results for the implants with screwed implant-abutment connection and the implants with cemented implant-abutment connection. The results are reported in Table 3.3.III and 3.3.IV.

Tab. 3.3.III: *Results of clinical exam for the Group 1 implants*

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 > 3mm;* 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.*

Tab. 3.3.IV: *Results of clinical exam for the Group 2 implants*

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	1	1	1	1	2	0	0	0	-	-	-
8	AN	4.6	1	1	2	1	2	1	1	1	2	-	-	-
9	JGD	4.6	0	1	1	0	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 > 3mm;* 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.*

The implants with screwed implant-abutment connection, Group 1, showed 14 sites of 60 that probed more than 3 mm (23%) with an average probing of 3.1 mm. The implants with cemented implant-abutment connection, Group 2, showed only 2 sites of 60 that probed more than 3 mm (3%) with an average probing of 1.8 mm (P=.0013; P<.05, Chi-test).

The sulcus bleeding was presented in 8 of 10 for the Group 1 implants (80%) and in 6 of 10 for the Group 2 (60%).(P=.04; P<.05, Chi-test)

Gingival alterations were evaluated in 6 cases of 10 (60%) for the Group 1 and in only 2 cases of 10 (20%) in the Group 2, but in either group were limited to edema.

The mechanical failures were found in two cases of 10 (20%) for screwed implant-abutment connection identified with the abutment unscrewing, and in one case of 10 for cemented implant-abutment connection identified with 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.

4. DISCUSSION

The precise mechanism responsible for the crestal bone remodeling in 2-piece implants is not known. One of the factors causing most concern is IAI (Implant-abutment interface). If the interface of the implant and abutment is not precise and does not fit adequately, it can have the formation of micro-gap. Microbial penetration through the micro-gap that 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 that, in long term, could seriously affect the health of peri-implant tissue.

According to Broggini e al., the precision of 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.[46] Rangert et al., McCartney et al., and May et al. also affirmed that the accurate assembly of implants 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.[47-49]

In rehabilitations with implants, external prosthetic connections of the external hexagon type, and internal connections, such as hexagonal, tapered (Morse Cone), or both in combination are basically used. Tapered connection appear to have superior stability when compared with the external hexagon type. According to Dibart et al., the frictional connection of a tapered pillar consists of a cold, metal-to-metal solder; creating sealing, making the IAI very narrow for the passage of bacteria.[50]

Prosthetic connections with better capacity to seal 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 cement-retained implant-abutments offer better results relating to fluid and bacterial permeability compared with screw-retained implant-abutments. Besimo et al. observed no contamination until 11 week at the internal surface of implants when chlorhexidine varnish was applied at the IAI, in internal hexagon connection;[51] however, Duarte et al. when assessing varnish containing 1% chlorhexidine and a silicone sealant, verified that these materials were incapable of preventing bacterial leakage.[29]

According to Quirynen et al., 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 accumulation of bacteria.[7]

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

However, it is very important to know about the factors, as well as their etiologies in determining bone loss.[13]

Hermann et al. concluded that radiographic and histologic analyses indicated that alteration at the bone crest depend on the characteristics of the implant surface, presence, absence and location of gap.[52]

Tonetti and Schimid conducted a literature review with regard to pathogenic processes that lead to osseointegration failures. The late failures that occurred after osseointegration was established 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 interaction between the host-parasite.[53]

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

The different connection systems were evaluated to three levels: in a *in vitro* study, with the aim to evaluate the bacterial leakage at the IAI; in a *computer model*, where by the FEA was analyzed the micro-motions and the stress distribution at the IAI; and in a *in vivo* study, where were compared the two implant systems evaluating the any clinical differences and the peri-implant micro-flora.

The results, in accordance with Schmitt et al., had showed the lower stability to the screwed implant-abutment connection than the cemented implant-abutment connection both for the permeability to the bacterial colonization than for the stability to the chewing forces.

In the *in vitro* study, the screwed implant-abutment connection had showed an higher colonization by bacteria than the cemented connection, with 100% implants colonized by bacteria versus 25%, and a more higher average total count (1,2E+07 versus 7,2E+04). The difference was statistically significant with a P value <.05 (Pvalue=.0001).

In the *computerized model*, not significant differences are showed between the two connection types. For either connections at the IAI is present a micro-motion between 0.09 and 0.87 µm and a stress between 82 and 141 MPa.

The higher micro-motion is evaluated at the level of Group 2 connection system, cemented connection, but it may be related to the impossibility, of the FEA simulation, to simulate the cement present into the fixture. Remains, so, a space between abutment and fixture that could be the cause of this result. The micro-motions are minimum at the level of the screw for the screwed connection because in this case the system was set with the tightening between internal screw and fixture.

In contrast, the stress is greater for the screw of the screwed implant-abutment connection with a result maximum of 36 MPa versus 0 MPa for the cemented abutment. This indicate that at the level of the internal screw there is an higher stress that in the time could to cause the unscrewing of the screw. The stress is not present in cemented abutment.

The fatigue analysis hasn't showed differences between the two implants, proving an high resistance of either system to the chewing forces, with a break only after more 1E+32 cycles.

The *in vivo* study, also, showed differences between the two implant systems. The peri-implant micro-flora was similar for the two implants, but the implants with screwed connection showed an high peri-implant sulcus colonization by periodontal-pathogenic bacteria, with a high risk of peri-implantitis and bone loss.

The greater differences, anyway, are proved by the clinical exam. The Group 1 implants, screwed connection, showed an higher number of sites with probing greater than 3 mm (14/60) than the Group 2, cemented connection, with only 2 sites on 60. The difference is statistically relevant with a P value <.05 (P value= 0.013).

Similar result is evaluated for the bleeding on probing. In this case the screwed implants showed a bleeding in 8 implants of 10 versus 6 of 10 for the Group 2. (P=.04; P<.05, Chi-test)

Gingival alterations were evaluated in 6 cases of 10 (60%) for the Group 1 and in only 2 cases of 10 (20%) in the Group 2, but in either group were limited to edema.

The mechanical failures were found in two cases of 10 (20%) for screwed implant-abutment connection identified with the abutment unscrewing, and in one case of 10 for cemented implant-abutment connection identified with 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.

All this results show that the screwed implant-abutment connection is weaker to resist to bacterial colonization and occlusal stress than cemented implant-abutment connection.

This suggest to choose the implant system with cemented abutment that seems to be more reliable in long term.

Naturally, for have a clear idea will be necessary to repeat this study with a larger population and for a more long term. Moreover will be appropriate to confirm this results with other studies also using load simulation systems.

At the end of this study, is possible, anyway, to conclude that, also if the implants long-term failures are consequences of multi-factorial elements, like hygiene, position of the implant, type of bone, systemic diseases, and more other, the choice of an adequate implant system is fundamental for the long-term success. In the light of the foregoing exposed, the choice of connection system is very important and will be preferable to choose implants with cemented connection instead of implants with screwed connection.

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