DENTAL IMPLANTS WITH LOCKING TAPER CONNECTION *VERSUS* SCREWED CONNECTION: MICROBIOLOGIC AND SCANNING ELECTRON MICROSCOPE STUDY

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The aim of this study is to carry out an analysis of the Fixture-Abutment Interfaces (FAI), comparing different connection systems, to evaluate the role of geometric discrepancy, which is present between the abutment and the fixture, in favoring the permeability to bacterial colonization. Two types of commercially available FAI were studied, 16 screwed FAI (Sweden-Martina Italia) (4 of Ø 3.8 mm, 4 of Ø 4.7 mm, 4 of Ø 5.7 mm and 4 of Ø 6.7 mm) and 4 FAI (Bicon) (Ø 3.5mm). The assays were carried out *in vitro*, placing the different dental implants in contact with broth culture of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* to test the infiltration inside the FAI. Furthermore, scanning electron microscope (SEM) analysis was carried out to evaluate the gap at the fixture-abutment interface. In all the locking taper FAI and in the screwed FAI with a diameter of 3.8 mm there was no trace of bacterial infiltration of the species examined. In the screwed FAI with a diameter of 4.7 mm, 5.7 mm and 6.7 mm there was an increasing level of bacterial infiltration in relationship to the diameter. Therefore, this paper shows that there exists an important correlation between the diameter of the screwed implant and the permeability to microbic infiltration that is directly proportional to the diameter of the implant.

In our society today tooth loss is seen as a defect that can compromise one's daily life and interpersonal, affective and working relationships. For these reasons patients with partial or total tooth loss usually undergo functional and esthetic implant prosthodontics, above all for the anterior region (1).

During the last decade the exponential increase in additive pre-implant surgery has given good results for the three-dimensional reconstruction of the maxillofacial complex, both in the cases of small bone defects and in cases of extensive atrophy (2-7). This progress has notably contributed to the diffusion and improvement of the surgical techniques used in the field of implants.

Over the last few years, implant research has concentrated on the characteristics of the components of the endosseous fixture, to establish the criteria for the evaluation of the clinical success of the implant based on a good bone integration (8-13). The attention of many researchers and clinicians is today focused on the study of commercially available connection systems, with the aim of avoiding some of the problems linked to implant failure. In fact, from a careful analysis of literature the most frequent causes of failure are due to bacterial inflammation (14-15); in implant-prosthesis the most feared complications, from the microbiological point of view, are perimplantitis and angular reabsorbtion.

One of the greatest risks of infection connected to two-phase implant systems is the presence of a geometric gap between fixture and abutment; this space can serve as a stagnating reservoir for a potent re-infection of the perimplant groove (16-17).

The aim of this study is to carry out an analysis on the Fixture-Abutment Interface (FAI), comparing different connection systems of dental implants, in order to evaluate the responsibility of the geometric discrepancy present at the fixture-abutment interface favoring the bacterial penetration in the internal part of different implants.

MATERIALS AND METHODS

Two types of commercially available Fixture-Abutment Interfaces (FAI) were studied: 4 types of screwed FAI (Sweden-Martina Italia) of different diameters, (FAI -a of Ø 3.8mm, FAI -b of Ø 4.7mm, FAI -c of Ø 5.7mm and FAI -d of Ø 6.7mm) a 1 type of locking paper FAI (Bicon) with diameter of Ø 3.5mm.

In this study 12 samples were used for each type of FAI.

Research was carried out in 2 steps as described below.

Bacterial penetration analysis.

Pure cultures of *Streptococcus pyogenes* ATCC 12344, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 were selected for the microbiological evaluation.

Suspension of the test organism was made from the 18 h culture by diluting a few colonies in BHI broth to a density of 0.5 McFarland Standard ($1x10^8$ colony forming units per ml [CFU/ml]).

Key words: dental implant, S.E.M., bacteria, taper connection, screwed connection

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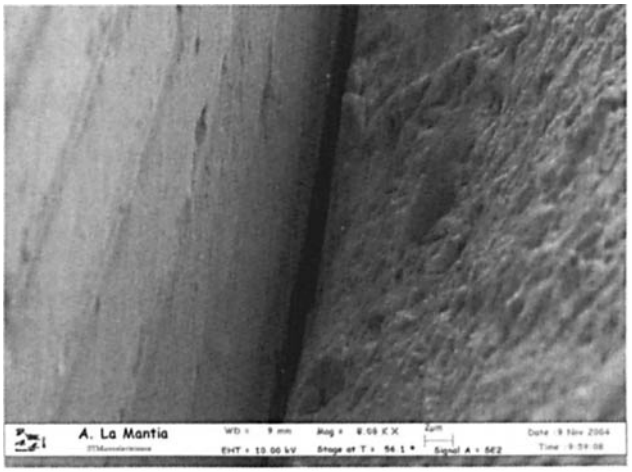

Fig. 1. *Locking taper FAI, enlarged 8000 X.*

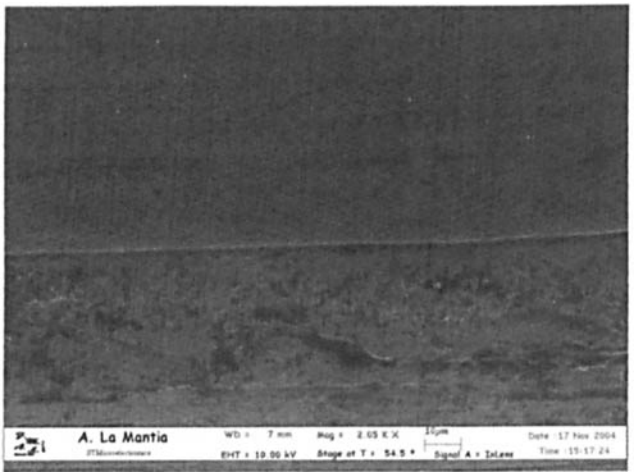

Fig. 2. *Screwed FAI –a, enlarged 2000 X.*

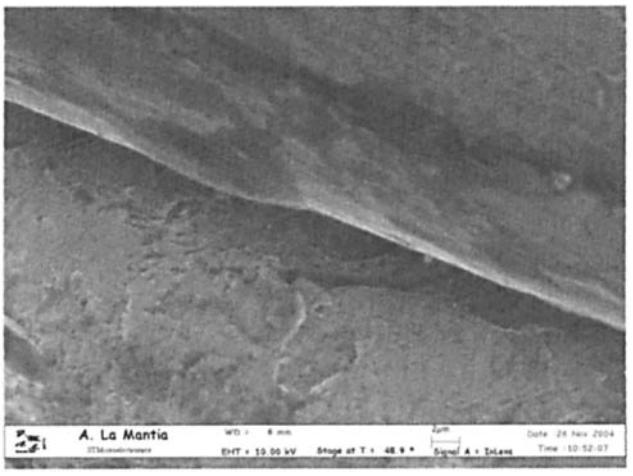

Fig. 3. *Screwed FAI –b, enlarged 3600 X.*

The two components of the tested connections, previoŭsly sterilized by UV rays, were assembled in sterile conditions after introducing 20 µl of sterile nutrient broth into the internal portion of the implant. Then the FAI were placed into 5 ml of the bacterial

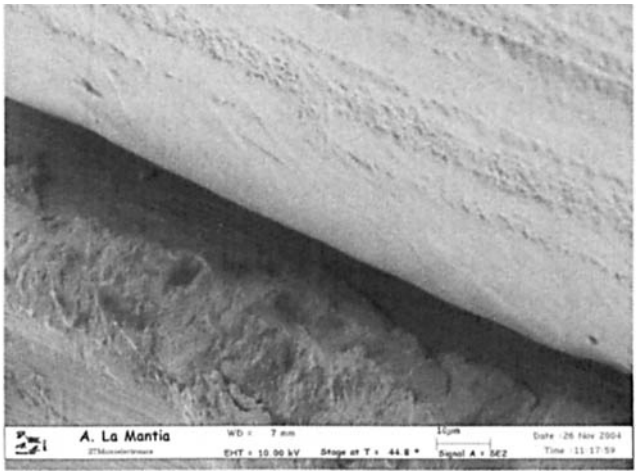

Fig. 4. *Screwed FAI –c, enlarged 3600 X.*

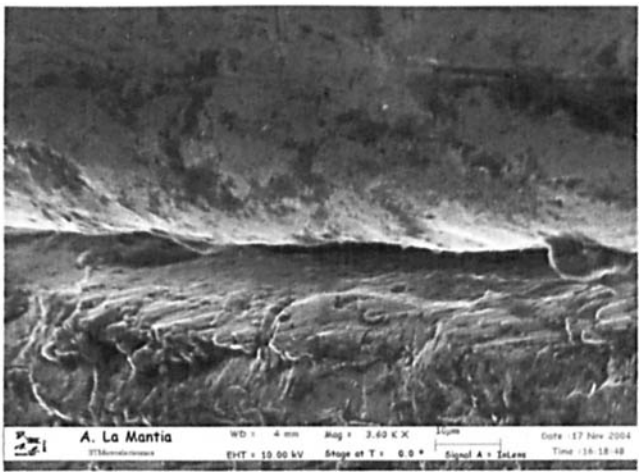

Fig. 5. *Screwed FAI –d, enlarged 3600 X.*

broth culture under examination so that only half of each implant (the abutment-connected part) was immersed in the bacterial suspension.

All the vials containing implants were placed in the rack of an agitator set to the speed of 60 cycles/minute and maintained at 37°C for 72 hours, replacing 50% of the bacterial suspension with fresh sterile nutrient broth every 24 hours. This was done to maintain an optimal vitality of the strain in the bacterial suspension in nutrient broth.

At pre-established time intervals (3, 6, 24 and 72 h) one sample of each type of FAI was removed, washed in sterile 0.1 M phosphate buffered saline at ph 7.0, disinfected with 0.2% cetrimide, rinsed with the same buffer and dried with sterile gauze. After separating the two parts of the connection the 20 µl of nutrient broth present inside the implants was removed and directly plated on Muller-Hinton agar to determine the total number of viable bacterial (CFU/ml).

For each sample, gram coloration and biochemical identification were performed to verify the purity of the colonies of the isolated and counted strain under examination.

Scanning electron microscopic analysis. To evaluate the presence of gaps between the fixture-abutment interface, SEM (Scanning Electron Microscope) analyses were carried out on the

Table I. *Bacterial recovery at the internal portion of 5 different fixture-abutment interfaces (FAI).*

	3h	6h	24h	72h
S. pyogenes ATCC				
screwed FAI –a	-	-	-	-
screwed FAI –b	-	-	-	-
screwed FAI –c	-	-	-	-
screwed FAI –d	-	-	-	-
locking taper FAI	-	-	-	-
S. aureus ATCC				
screwed FAI –a	-	-	-	-
screwed FAI –b	-	-	7.1×10^3 CFU/ml	0.3×10^4 CFU/ml
screwed FAI –c	-	-	6.5×10^3 CFU/ml	0.1×10^4 CFU/ml
screwed FAI –d	-	5.0×10^2 CFU/ml	2.0×10^4 CFU/ml	7.5×10^4 CFU/ml
locking taper FAI	-	-	-	-
P. aeruginosa				
screwed FAI –a	-	-	-	-
screwed FAI –b	-	-	-	-
screwed FAI –c	-	0.7×10^2 CFU/ml	1.1×10^4 CFU/ml	5.0×10^4 CFU/ml
screwed FAI –d	-	-	-	-
locking taper FAI	-	-	-	-
E. coli				
screwed FAI –a	-	-	-	-
screwed FAI –b	-	-	-	-
screwed FAI –c	-	-	2.2×10^4 CFU/ml	5.0×10^4 CFU/ml
screwed FAI –d	-	-	-	-
locking taper FAI	-	-	-	-

different types of FAI used for the microbic study.

The fixtures and the abutments were received in their original, sterile packing, which was only opened at the moment of SEM analysis. The FAI were analyzed without undergoing any particular preparation procedure.

For the analysis of geometric discrepancy at the level of the contact surface between the fixture and abutment, a low voltage scanning electron microscope (SEM.), (LEO 1550 F.E.) was used with a Gemini column; this microscope allowed the non-destructive description of the samples without any covering, and obtained an extremely high resolution, which varied from 3 nm (at 1 kilovolt) to 1 nm (at 30 kilovolt), at a working distance (WD) of 2 mm. The samples were examined at various enlargements, range 100X to 3000X.

The technical specifications and reference parameters for the electronic microscope were as follows: beam acceleration tension (EHT), which in all the tests was EHT=20.00 Kv; electron beam current (Probe Current) was 40 pA; the resolution was 1nm at 20 Kv for a WD=2mm; tilt angle was Tilt=0 degrees.

For all the samples analyzed, the Secondary Electron Images (SE2) were used as detectors for material characteristics.

The measurement of the gap was carried out using the following measurement program: *Point to Point measure μm.*

RESULTS

Bacterial penetration analysis. The results obtained from the microbic study on the locking taper and screwed FAI are shown in Table I.

In all the tests carried out there were no cases of accidental bacterial contamination.

In the locking taper FAI and in the screwed FAI -a, there was no fluid penetration bacterial growth at 3, 6, 24 and 72h for all the strains assayed.

After 3 h there was no infiltration in all the FAI types, both screwed and locking taper connection. After 6 h from inoculation there was contamination by *S. aureus* only for the FAI -d (5.0×10^2 CFU/ml) and by *P. aeruginosa* only for the FAI -c (0.7×10^2 CFU/ml).

After 24 h the results show a contamination by *S. aureus* for the FAI -b (7.1×10^3 CFU/ml), for the FAI -c (6.5×10^3 CFU/ml) and for FAI -d (2.0×10^4 CFU/ml); *P. aeruginosa* and *E. coli* were able to contaminate, by means of infiltration, only the FAI -c (1.1×10^4 CFU/ml and 2.2×10^4 CFU/ml, respectably).

No growth of *S. pyogenes* was observed in any hollow portion of the tested FAI.

Scanning electron microscope analysis. From the SEM analysis at various magnifications it was seen that there were different geometric discrepancies at the fixture-abutment implant interface.

The locking taper FAI had a geometric gap of between 0.5 and 1.5 μm (Fig. 1);

DISCUSSION

The aim of this study is to evaluate the responsibility of the geometric discrepancy present between the fixture-abutment, in favoring the bacterial penetration in different connection systems of dental implants.

The results show that an important correlation between the diameter of the screwed implant and the permeability

to microbic colonization, in as much as the increase in interface diameter corresponds to an increase of the bacterial load that can leak from the connection.

It should be noted that the *in vitro* conditions studied are much more favorable for microbial growth than those *in vivo* and moreover, the growth of the bacteria was not hindered, as physiologically happens, by the presence of enzymes, by mechanical removal or by competition between microbes of different species.

The results showed that neither fluid penetration nor bacterial growth of the tested microorganisms was observed after 72 h inside locking taper FAI and in the screwed FAI -a. This finding is supported by a minimum gap in the locking taper FAI (0.5 – 1.5 μm) and in the FAI –a (1.5 – 2.0 μm), found after SEM analysis, which hinders the microbic passage in both directions.

The bacterial infiltration in the screwed FAI -c and FAI -d and the reproduction kinetics were higher than with FAI -b. This finding is confirmed by the different gaps (4 – 10 μm) that exist between the implant and the abutment in the screwed FAI with a diameter greater than 3.8 mm.

Several authors (16, 18-21) observed bacterial colonization of the internal part of dental implants. Moreover, the existence of microbial leakage along the components of the Brånemark® implants system was examined *in vitro* (21); a similar observation was reported (22) for another system. The clinical importance of this bacterial leakage is not yet well understood .

The studies of these authors confirm our results that show that the penetration of *S. pyogenes* ATCC 12344, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 (strain with dimensions ranging from 0.5 to 3 μm) from the bacterial suspension to the internal portion of the implant was caused by a gap in the implant-abutment junction.

Other *in vivo* studies found that bacteria inside the implants were similar to those found in root canal infected pulp chambers, and deep periodontal pockets. The presence of bacteria inside the implants could produce an inflammation of the peri-implants tissues, and this could interfere with the long-term success of the implants, (23), reported that the gap in submerged implants had a significant effect on whether there is an active or subacute clinical inflammation.

Our results demonstrated that *in vitro* no fluid or bacterial growths were found inside the locking taper FAI and the screwed FAI –a, but that penetration of bacteria may occur from an external source inside the other screwed FAI (-b and -d).

On the basis of the results obtained in the present study it is clear that the locking taper FAI and the screwed FAI -a are valid mechanical barriers for bacterial infiltration, impeding the eventual inflammation of peri-implant soft tissues or angular bone reabsorbtion, which can be the cause of the loss of the implant itself.

Given the importance of this research, further studies are underway to evaluate the efficacy of the above mentioned FAI, based on medium and long-term implant survival.

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