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original research article

IMPLANT DENTISTRY: MONITORING OF BACTERIA ALONG THE TRANSMUCOSAL PASSAGE OF THE HEALING SCREW IN ABSENCE OF FUNCTIONAL LOAD

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SUMMARY
Purpose. To assess the changes in bacterial profile along the transmucosal path of healing screws placed immediately after insertion of two-piece endosseus implants during the 4-month osseointegration phase, in absence of functional load.
Materials and methods. Two site-specific samples were collected at the peri-implant mucosa of the healing screws of 80 two-piece implants, for a total of 640 samples. Implants placement was performed following a single protocol with flapless technique, in order to limit bacterial contamination of the surgical site. Identical healing screws (5 mm diameter/4 mm height) were used for each of the 80 implants. During the 4 months of the study, the patients followed a standard oral care regimen with no special hygiene maneuvers at the collection sites.
Results. The present research documents that during the 4-month period prior to application of function load the bacterial profile of all sites exhibited a clear prevalence of cocci at the interface between implant neck and osteoalveolar crest margin.
Conclusions. A potentially pathogenic bacterial flora developed only along the peri-implant transmucosal path.

Key words: bacterial morphological profile, transmucosal peri-implant path, healing screw, two-piece implant, absence of functional load.

Introduction

Dental plaque is the accumulation of organic and inorganic mixed material with a significant microbial content, especially bacteria, which – in addition of depositing on the teeth surface – can also adhere tenaciously to implant fixtures (1-3).
Previous studies underscored the similarities in composition between the plaque found on teeth and that found on implant abutments. The latter type is mostly made up of Gram-positive aerobic cocci and non-motile bacteria (4, 5).
In experiments on dogs, Berglundh and Ericsson (6, 7) found existing similarities between the amount and composition of the plaque formed on teeth and that observed at implant sites. Implants as well as teeth surrounded by healthy mucosa, are invariably associated with a plaque composed mainly by cocci and Gram-positive bacteria while the sites affected by diffused periodontal and peri-implant disease exhibit biopellicles rich in rod-shaped, fusiform and spiral

bacteria, which – in addition of being motile bacteria – greatly outweighs the number of cocci (8, 9).
The above corroborates the results of Listgarten, according to whom the proportion of cocci found at the implant site corresponds to 71.3% of the total bacteria, while other types of bacteria are a minority, especially the non-motile strains (0.4%) (10). These data differ significantly in those sites with periodontal and peri-implant inflammation, where the percentage of cocci is markedly decreased. The aim of the study was to evaluate the bacterial profile from a morphological standpoint, and the ratio of the different types of bacteria found along the transmucosal path of healing screws placed after insertion of two-piece fixtures during the 4-month osseointegration phase in absence of load.
Specifically, the research aimed at assessing the differences in bacterial composition of the plaque accumulated on the mucosa surrounding titanium healing screws, before insertion of the definitive abutment and application of the functional load (Figure 1).

Materials and methods

All implant placements were performed following the same general protocol and flapless technique, in order to limit bacterial contamination of the surgical site. At the edentulous sites, complete perforation of the attached mucosa was achieved by means of a 4.1 mm Ø tissue punch. Identical healing screws (5 mm Ø, height 4 mm) were used for each of the 80 implants.
Starting the month following implant placement, each site was followed-up for four months, a period corresponding to the so-called osseointegration phase, under non-functional loading conditions.
The bacterial plaque accumulated on the 80 implants was collected on a monthly basis, for a total of 640 samples at the two considered sites:
1) along the transmucosal path of the healing screw;
2) at the interface between implant neck and osteoalveolar crestal margin.

Each collected sample was submitted to morphological assessment of the non-fixated vital bacteria by means of a technique employed by our study group for over 20 years, and following Listgarten's principles, according to whom a bacterial profile composed mainly of cocci (70%) should be regarded as normal and saprophytic compared to a high percentage of pathogenic bacteria such as rod-shaped, fusiform and spiral types (11).
The samples were labeled as follows:
TMP: sample collected along the transmucosal path;
CMI: sample collected at the crestal margin interface.
The first collection phase was performed with a sterile point along the transmucosal path (TMP), leaving the healing screw and its bacterial film (plaque) in place to avoid alteration of the microbiota conditions at the different levels of the collection site (Figure 2).

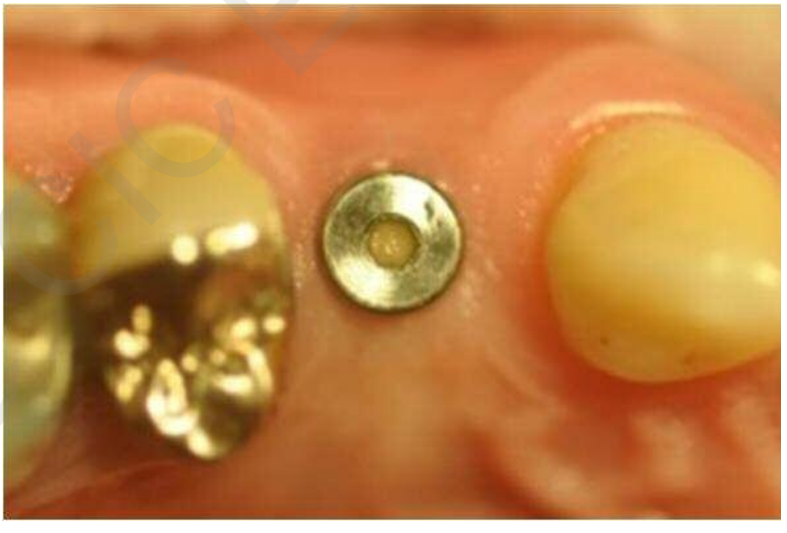


Figure 1
Healing titanium screw of one of the 80 cases analyzed.

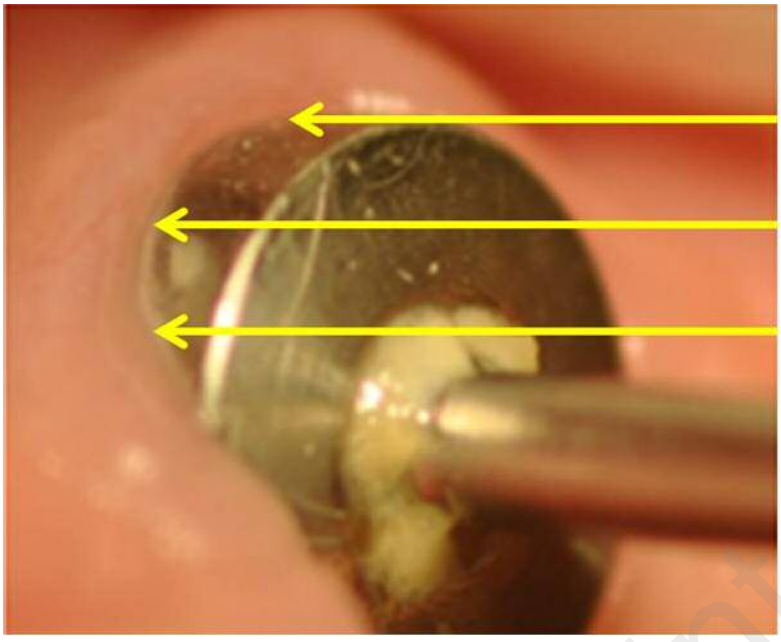


Figure 2
The first phase of withdrawal was performed using a sterile tip in scope of the transmucosal route (TMP), indicated by the arrows, while maintaining the healing screw *in situ* with the presence of its bacterial film (plaque) does not vary the conditions for microbial between the various levels of the levy niche.

The second collection phase (CMI) was performed *after removal of the healing screw*, taking care to avoid contamination of the sterile tool used for sampling along the transmucosal path, characterized by abundant plaque (Figures 3-5). The healing screw was then repositioned to preserve the environmental conditions for the three following monthly collections.
The removed healing screws have constantly exhibited a significant amount of bacterial plaque on their entire surface (Figure 6). A massive presence of plaque was also found on the inner surface of the fixture, despite the presence of the healing screw (Figure 3).
The bacterial morphology assessment of the vital intact bacteria was carried out by means of contrast-phase microscopy (Figure 7).
The material collected with a sterile point from the sample sites (TMP – CMI) was diluted with 1% lyophilized gelatin in saline solution.
With a variable volume micropipette (Socorex – Isba S.A.-Switzerland), 1000 µL of solution were withdrawn and dissolved in the master vial (MV).
The MV underwent dynamization by agitator heatable magnetic (I.S.Co.,Mod. AMR 2T-Italy) so as to obtain an uniform distribution of the sample in the test vial.
The sample was then allowed to rest for 3 minutes.
With a new sterile tip on the micropipette, 100 µL of

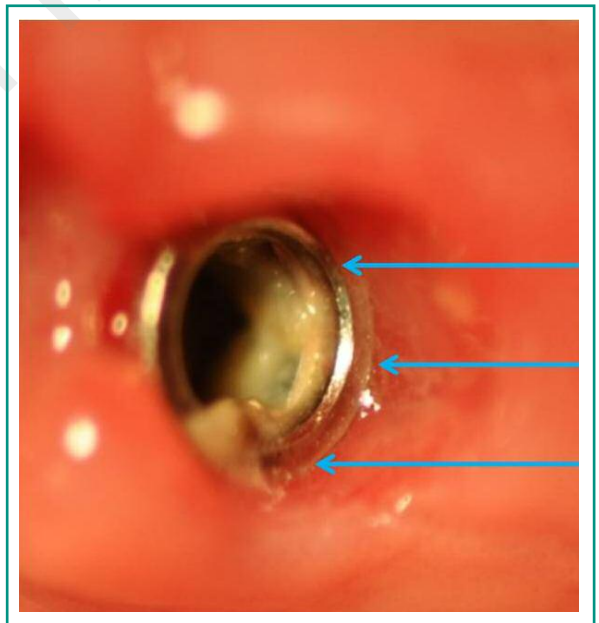


Figure 3
Second phase of withdrawal (CMI): blue arrows show the margin-alveolar crestal bone after the healing screw removal. Observe the amount of plaque.

diluted sample were transferred in a derived vial (DV) containing 900 µL of saline, thus obtaining a 1:10 dilution ratio. This vial also underwent dynamization similarly to the MV so as to achieve a homogeneous

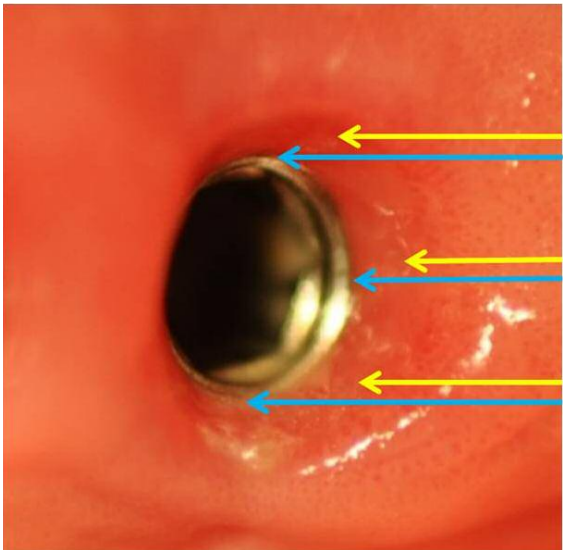


Figure 4
The arrows indicate the sampling sites. The transmucosal route TMP (yellow arrows) and the margin alveolar crestal bone-CMI (blue arrows).

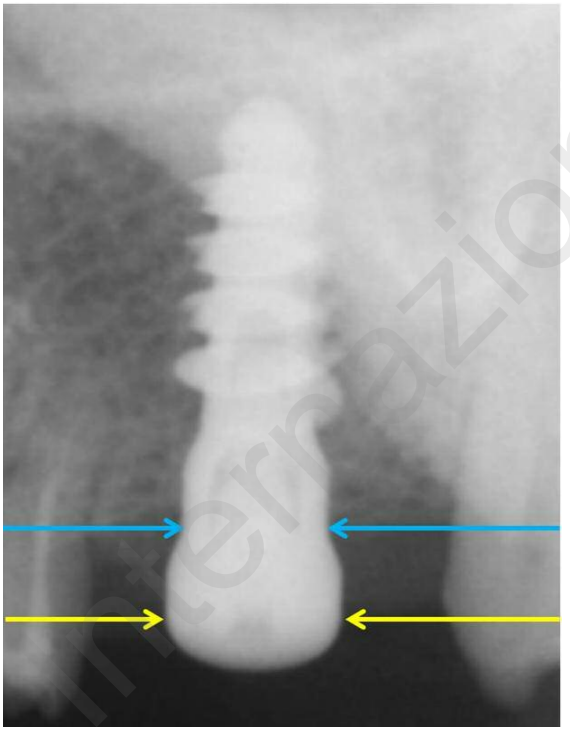


Figure 5
Position provides amply TMP (yellow arrows) and margin-alveolar crestal bone IMC (blue arrows) seen in the image X-ray.

dispersion of the bacterial content, and the sample was allowed to rest for 3 minutes.
Subsequently, 100 µL of diluted sample were withdrawn from the vial, deposited on the surface of a Bürker chamber, and covered with a coverslip.
The slide was then examined at 40x with a contrast-phase microscope.

Reading was performed for every surface unit (1/25 mm^2) proceeding from top to bottom and left to right and the results were noted on an *ad hoc* table.

Figure 6
The plaque adherent to the surface of the titanium healing screw.

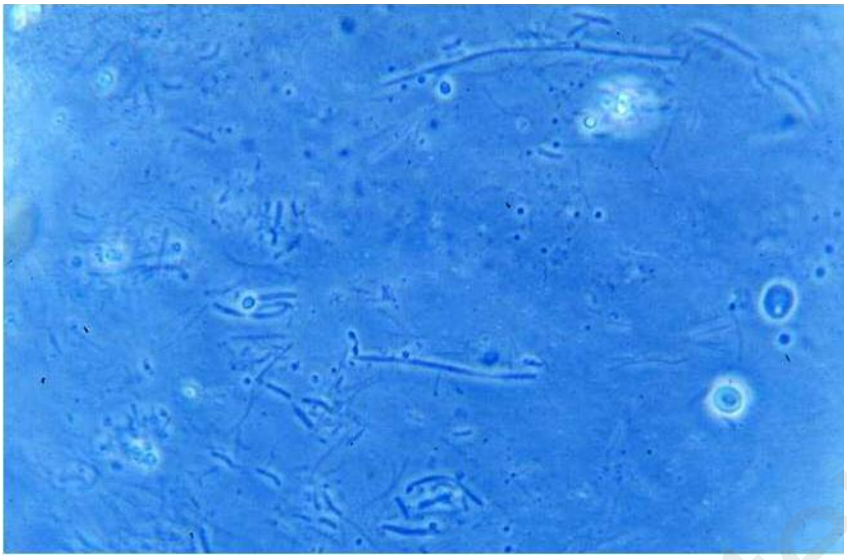


Figure 7
40x microscope view of the levy of TMP provides amply when you notice the presence of all bacterial morphology (cocci, rods, filaments and convoluted).

Results

Results are summarized in Table 1. The data show a significant evolutionary differentiation in terms of percentage of cocci in the samples collected at the TMP compared to the CMI sites, during the 4 months of the study. The progressive reduction of the number of cocci at the TMP sites in favor of other forms of bacteria indicates that the microbiota niche can be easily colonized by bacterial plaque. At the IMC sites, the presence of cocci remains practically constant and they are clearly more prevalent than other bacterial types, meaning that the overall the echo-microbiota conditions are unfavorable to the growth of rod-shaped, fusiform and spiral-type bacteria.

Due to the fact that the results were expressed in percentages, the analysis was performed with non-parametric methods (12, 13).

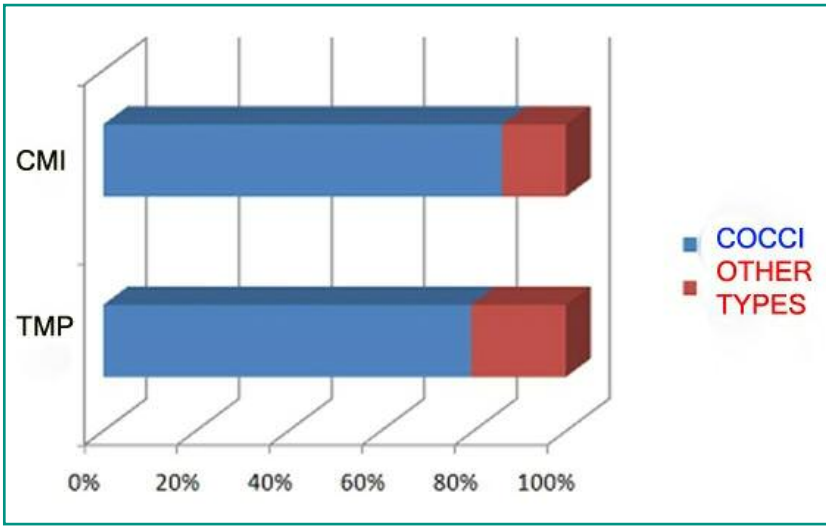
The Mann-Whitney test was used to assess the statistical significance of the differences between the two simultaneous sample collections (TMP - CMI) (14).

Therefore, for the four timepoints, the TMP samples show a highly significant reduction of cocci compared to the IMC samples.

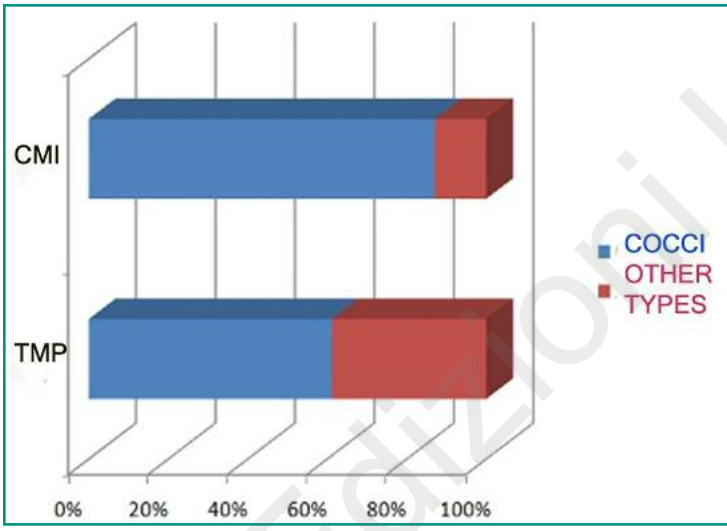
The Kruskal-Wallis test was used to assess the statistical significance of the variation at the different timepoints, for each site (15) (Graphics 1-4).

Table 1 - Percentage of cocci in the samples collected at the TMP compared to the CMI sites, during the 4 months of the study.

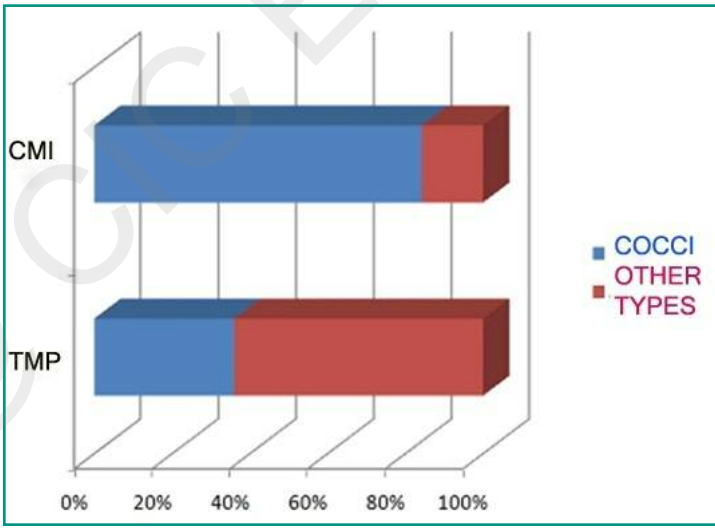
Bacteria morphological examination percentage of cocci				
Collection	**TMP**		**TMC**	
	n	**Average %**	**n**	**Average %**
MONTH 1	80	79.51	80	86.24
MONTH 2	80	61.20	80	87.11
MONTH 3	80	36.09	80	84.36
MONTH 4	80	25.83	80	85.64



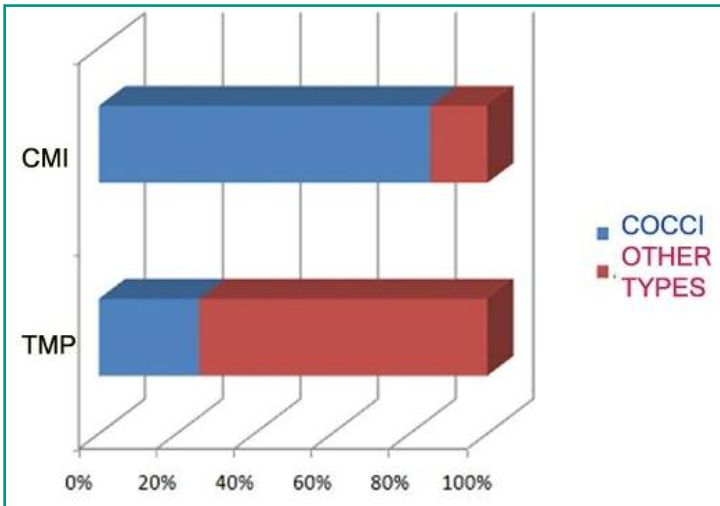
Graphic 1
One-month follow-up. The differences in bacterial loading in CMI and TMP are statistical significant by using Mann-Whitney U test.



Graphic 2
Two-month follow-up. The differences in bacterial loading in CMI and TMP are statistical significant by using Mann-Whitney U test.



Graphic 3
Three-month follow-up. The differences in bacterial loading in CMI and TMP are statistical significant by using Mann-Whitney U test.



Graphic 4
Fou-month follow-up. The differences in bacterial loading in CMI and TMP are statistical significant by using Mann-Whitney U test.

The above shows that the progressive reduction of cocci along the transmucosal path is highly significant, while the interface with the crestal margin remains pretty much unchanged and has no pathogenic significance (10).

Discussion

The research aims to highlight that in the absence of occlusal loading and in particular of environmental stressors (dysfunctional occlusal loading) the peri-implant tissue at the crestal margin undergoes spontaneous stabilization of the bacterial profile (16-19). The orderly and relatively repetitive presence of Gram+ cocci prevents the development of peri-implant pathogenic microbial conditions conducive to bacterial invasion of the deeper tissues which can cause infection followed by peri-implantitis (20, 21).
The ecosystem relationships between host organism and bacteria are therefore well maintained in terms of integrated ecological component (microbioma) (22, 23).
The above mentioned evaluation are of paramount importance especially in patients with oral disease (24-33).
The absence of biomechanical stress (occlusal-static-dynamic load), permits maintenance of a balance between the system consisting of implant screw-bone-mucosa on one side and bacterial front on the other side.
Bacterial component maintains a non-invasive/aggressive saprophytic-commensal profile, regardless of the types of bacteria that progressively develop along the transmucosal path (TMP) and at the interface implant-crestal bone margin (CMI).
This study shows that in a multifactorial biological context in non-loading conditions, a massive presence of bacteria is not sufficient *per sé* to have a destabilizing effect on the balance between host and bacterial front (34-41).
The dysfunctional load – i.e. occlusal trauma – could be a predisposing factor able to trigger the change in bacterial profile from saprophyte to pathogen (16-19).
Peri-implant disease could represents a biomechanic dysfunctional based syndrome whom damage it's mainly atrophyc-distrophyc. Talking about implant-protesis, this pathological manifestation (peri-implantitis) it becomes stronger due to the lack of those organs of functional manifestation with a modulated distribution of loads, in fact the periodontal ligament, avoiding the contact between the root surface and the bone, represents the optimum biomechanical condition, even if is impossible in case of implant-bone ankylosis. However, unfortunately implant failure is often attributed to microbial causes, systemic diseases, inadequate hygiene and / or smoking (42-79).

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