Vol. 10, No 1 (S2), January - April 2012

ISSN 1721-727X

IMPACT FACTOR 4.000

European Journal of Inflammation

Supplement

Official Journal of the Italian Society of Oral Pathology and Medicine

Published by
Biolife

www.biolifesas.org

SEM EVALUATION OF 10 INFECTED IMPLANTS RETRIEVED FROM MAN

G. BRUNELLI[1], F. CARINCI[2], I. ZOLLINO[2], V. CANDOTTO[2], A. SCARANO[3], D. LAURITANO[4]

[1]*Dental Clinic, University of Cagliari, Cagliari, Italy*
[2]*Department of D.M.C.C.C., Section of Maxillofacial and Plastic Surgery, University of Ferrara, Ferrara, Italy*
[3]*Department of Oral Science, Nano and Biotechnology University of Chieti-Pescara*
[4]*Department of Neurosciences and Biomedical Technologies, Dental Clinic, University Milano Bicocca, Milan, Italy*

Peri-implant disease represents a collective term to describe inflammatory reactions in the tissues surrounding an implant. Results from clinical and experimental studies revealed that the tissue response to plaque formation at teeth and dental implants is similar. However, while peri-implantitis and periodontitis have many clinical features in common, structural differences in supporting tissues between implants and teeth may influence host response to infection. Here a SEM evaluation was reported to evaluate quality of bacteria and the pertinent literature discussed. Ten implants had to be removed for progressive marginal bone loss during follow-up period. The implants surface was examined under a Scanning Electron Microscopy (SEM LEO, Cambridge, England) with tilt angles ranging from 10 to 45 degrees. SEM evaluations were performed by three independent observers who expressed an estimate of bacterial amount of three different areas: supra-crestal, sub-crestal and screw threads. Plaque formation and gingival inflammation were observed into the junctional epithelium-to-implant contacts, with also active or previous bone resorption. In peri-implantitis the implant surface facilities the adherence of the biofilm bacteria and complicates its elimination. Most of chemical and mechanical devices are not able to completely remove bacteria from implant surface especially if they are enclosed in calcified areas. Bacteria determine an inflammatory process which determines bone resorption around fixtures and thus implant mobility occurs. Identification of bacteria types is of paramount importance in order to perform specific therapy to eliminate peri-implant colonies.

The term periodontal disease usually refers to the common inflammatory disorders of gingivitis and periodontitis that are caused by pathogenic microflora in the biofilm or dental plaque that forms adjacent to the teeth on a daily basis (1). As the gingivitis refers to a gingival inflammation with no signs of supporting tissues loss, the periodontitis in addition to gingival inflammation is characterized by loss of attachment and bone (2-6).

Results from clinical and experimental studies revealed that the tissue response to plaque formation at teeth and dental implants is similar. The inflammatory lesions that develop in the tissues around implants are collectively recognized as "peri-implant diseases". In accordance with the classification of periodontal disease at teeth, peri-implant disease includes two entities: *peri-implant mucositis* that corresponds to gingivitis and *peri-implantitis* that corresponds to periodontitis. The definitions of the two peri-implant disease entities were proposed in a consensus report at the 1st European Workshop on Periodontology (7).(EWOP) However, while peri-implant mucositis was defined as a reversible inflammatory reaction in the soft tissues surrounding a functioning implant with no signs of supporting bone loss, peri-implantitis, in addition to the muscosa inflammatory

Key words: Implant, fixture, inflammation, bone, resorption.

Corresponding author: Prof. Francesco Carinci, M.D
Department of D.M.C.C.C.
Section of Maxillofacial and Plastic Surgery
University of Ferrara
Corso Giovecca 203 44100 Ferrara Italy
E-mail: crc@unife.it Web: www.carinci.org
Phone: +39.0532.455874 Fax: +39.0532.455876

0393-974X (2012)

reactions, is associated and is characterized by loss of supporting bone around an implant in function (2). Detection of inflammation in the peri-implant mucosa requires the use of periodontal probing to identify bleeding and/or suppuration following such a mechanical challenge. For the assessment of peri-implantitis, radiographs are needed to marginal bone loss detection. In this context, it is important to distinguish between the bone remodelling that occurs early after implant installation and the loss of supporting bone that may be detected at implants during function, i.e. after that process of osseointegration is completed (2).

Histological characteristics of peri-implant mucositis and peri-implantitis lesions were analysed in human biopsies. It was reported that the inflammatory cell lesion in sites with peri-implant mucositis was dominated by T cells and had an apical extension that was restricted to the barrier epithelium (6). In peri-implantitis the lesion extended apical to the pocket epithelium and contained large proportions of plasma cells and lymphocytes but also PMN cells and macrophages in high numbers (8, 9).

Peri-implant disease represents a collective term to describe inflammatory reactions in the tissues surrounding an implant. However, while peri-implantitis and periodontitis have many clinical features in common, structural differences in supporting tissues between implants and teeth may influence host response to infection. Analysis of the two types of lesions is important in the assessment of diagnosis and in the planning of peri-implantitis treatment protocols (10). By analogy to the aetiology of periodontitis, the pre-requisite and pivotal aetiological factor for the development of peri-implantitis is microbial colonization in the form of microbial plaque biofilms (5, 11). A statistically significantly higher incidence of peri-implantitis for implants placed in patients with a history of chronic periodontitis (28.6%) compared with periodontally healthy subjects (5.8%) has been reported (11). Additionally, an association between periodontal and peri-implant conditions has been demonstrated for the same population (11). Two recent systematic reviews (12, 13) came to the conclusion that implants placed in patients with a chronic periodontitis history may demonstrate a higher incidence of peri-implantitis than implants placed in patients without such a history; thus, the history of chronic periodontitis may pre-dispose to the development of peri-implantitis.

In light of the aforementioned evidence and given the continuously increasing number of implants placed in everyday clinical practice, it is reasonable to anticipate an increasing prevalence of peri-implantitis, which underlines the necessity for a predictable therapy (11).

Here a SEM evaluation was reported to evaluate quality of bacteria and the pertinent literature discussed.

CASE REPORT

Ten implants had to be removed for progressive marginal bone loss during the follow-up period. All the retrieved implants were mobile and were surrounded by a radiolucent line on radiographs. They were retrieved under local anesthesia by gently unscrewing them with stainless steel forceps. In order to avoid any possible contamination of the implant surfaces the stainless steel forceps were carefully positioned on the cover screw or on the abutment. The implants were rinsed with sterile physiological saline (NaCl) solution and were immediately immersed in such a solution contained in plastic vials used for transporting tissue samples for histopathologic examination. Special care was taken to avoid any possible source of contamination.

The implant sites were carefully curetted from remaining soft tissue and flaps were raised to achieve primary closure. The failed implants were retrieved from 3 years up to 8 years after loaded .

Scanning Electron Microscopy

After removal, the implants were rinsed three times in 15 mL DI water to remove non-adherent bacteria. Subsequently, they were dried in air for 5 minutes and they were put in 2.5% Glutaraldheyde with 0.1 M Sodium Cacodylate buffer at pH 7.4 for 4 hours. Then, they were dehydrated with increasing concentrations of ethanol solutions (50-70-90-100%) and left for 12 hours in 113 Freon (Trichlorotrifluoroethane) as a transition fluid to Critical Point Drying (CPD) Bomb Polaron. The chambers were, finally, glued to aluminum stubs and coated with 20-30 nm of gold. The implants surface was examined under a Leo 435VP Scanning Electron Microscopy (SEM LEO, Cambridge, England) operating at 20-30 KV with tilt angles ranging from 10 to 45 degrees. SEM evaluations were performed by three independent observers. They expressed an estimate of bacterial amount of three different areas: supracrestal, subcrestal and screw threads. Five areas of 100-130 microns in diameter were evaluated for each screws and a JPEG format image was created. Ten digital images have been used per region. The same images were evaluated by the three observers. Each image was evaluated twice, two days apart by each of the examiners. Intra-examiner and inter-examiner duplicate measurements showed no significant differences ($p=0.743$). The percentage of surface covered by bacteria was carried out on the JPEG images using a PC (Intel Pentium III 1200 MMX). This PC was associated with a histometry software package with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc Milano, Italy).

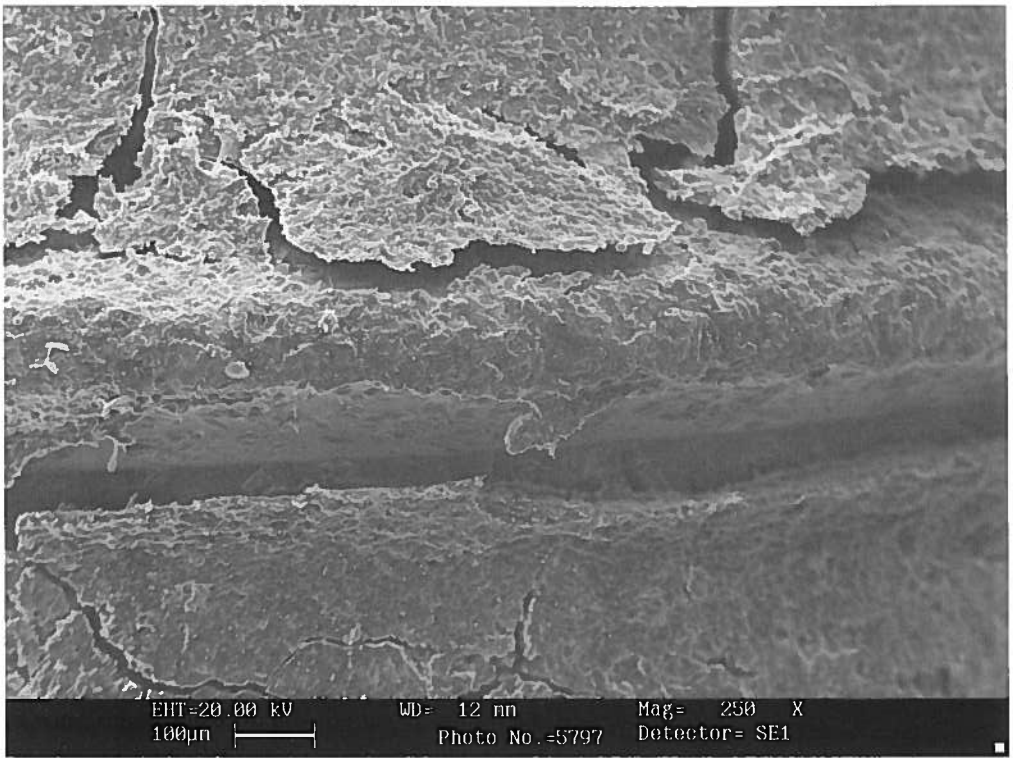

Fig.1. *The photomicrograph (a mag. 250x) shows an implant failing due to bacterial plaque contamination of the surfaced*

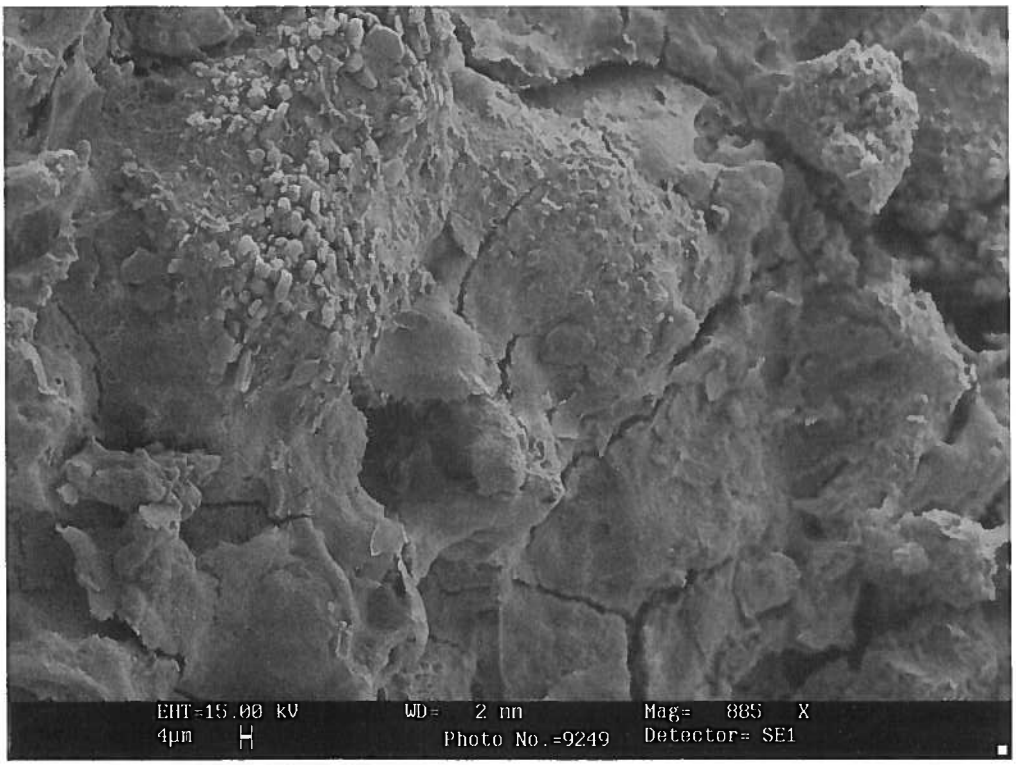

Fig.2. *This photomicrograph (a mag. 855x) shows heavy plaque deposits on the implant surface and the associated leukocytic exudates.*

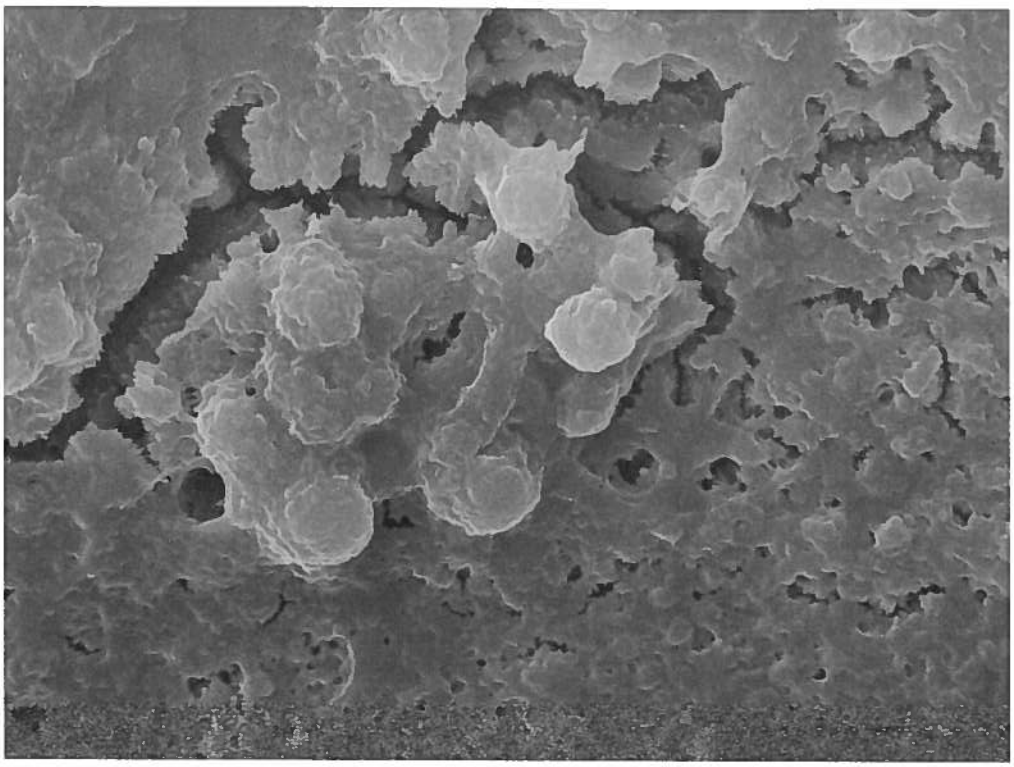

Fig.3. *This photomicrograph (a mag. 1200x) shows many area of calcification of the bacteria was observed*

RESULTS

Plaque formation and gingival inflammation were observed into the junctional epithelium-to-implant contacts, with also active or previous bone resorption. The plaque was even observed, consisting of few cocci and a higher proportion of rods and filamentous-shaped bacteria.

A thin and regular layer of cocci was found in many areas of the implant surface. Salivary proteins, in contact with implant surface, were observed. The salivary proteins were identified because they had an irregular shape and they were smaller than bacteria. At higher magnifications, salivary proteins, cocci, and many colonies of microorganism were found. A big area of bacteria calcification was present (Fig. 1-3).

DISCUSSION

Over the last decades, dental implants have become a commonly used treatment alternative to other dental procedures. The prognosis of implant therapy in dentistry is perceived to be very good (14).

Nevertheless, infections adjacent to implants occur. Thus, the peri-implant mucositis term was proposed to represent the reversible inflammation of the soft tissues surrounding implants, and if such an inflammation is combined with loss of bone, it is referred to as peri-implantitis (7). Peri-implantitis, if not successfully treated, may lead to complete disintegration and implant loss (14).With an increasing population with dental implants, the prevalence of implant-related infections would most likely increase and cause major challenges to therapy (14).

The goal in non-surgical therapy of peri-implant mucositis and peri-implantitis is to eliminate or significantly reduce the amounts of oral pathogens in the pockets around implants to a level that allows healing and re-establishment of a clinically healthy condition. However, using conventional means of therapy, eradication of pathogens by mechanical means on implant surfaces with threads and often with rough surface structures is difficult (15). Treatment models, such as scaling and root planning, effectively used to treat teeth with periodontitis, cannot be used in the same way on rough threaded implant surfaces (14).

Karring et al. (16) showed that sub-mucosal debridement alone, accomplished by utilizing either an ultrasonic device or carbon fibre curettes, is not sufficient for the decontamination of the surfaces of implants

with peri-implant pockets >5 mm and exposed implant threads. Thus, because mechanical debridement alone appeared to be insufficient for the decontamination of implant surfaces, it was considered rational to examine the efficacy of the adjunctive use of chemical antiseptic agents for non-surgical therapy of peri-implantitis. A study by Schwarz et al. (17) demonstrated that the treatment of peri-implant infection by mechanical debridement with plastic curettes combined with antiseptic therapy may lead to statistically significant improvements in bleeding on probing. However, Renvert et al. (18) proved that the adjunctive benefits derived from the addition of an antibiotic to mechanical debridement tend to be greater, although to a limited extent, than those achieved by the combined use of an antiseptic (chlorhexidine) and mechanical debridement. The addition of antiseptic therapy to mechanical debridement does not provide adjunctive benefits in shallow peri-implant lesions, (18) but seems to provide additional clinical improvements in deep peri-implant lesions (17).

A clinical trial by Romeo et al. (19, 20) concluded that resective surgical procedures coupled with implantoplasty could have a positive influence on the survival rates of rough-surfaced implants affected by peri-implantitis as well as on peri-implant clinical parameters, such as PPD, suppuration and sulcus bleeding.

In conclusion, peri-implantitis therapy comprises the non-surgical phase, which includes debridement by mechanical means, ultrasonic or laser devices, either alone or combined with antiseptic and/or antibiotic agent and the surgical phase, utilizing either resective or regenerative techniques (11). However, until now, no methodology has been established as a gold standard approach for the treatment of peri-implantitis disease (11).

Here a SEM evaluation on 10 dental implants was reported: a thin and regular layer of cocci was found in many areas of the implant surface. Salivary proteins, in contact with implant surface, were observed. At higher magnifications, salivary proteins, cocci, and many colonies of microorganism were found. A big area of bacteria calcification was also present.

Most of chemical and mechanical devices are not able to completely remove bacteria from implant surface especially if they are enclosed in calcified areas. Bacteria determine an inflammatory process which causes bone resorption around fixtures and thus implant mobility occurs. Identification of bacteria types is of paramount importance in order to perform specific therapy to eliminate peri-implant colonies.

ACKNOWLEDGEMENTS

This work was supported by FAR (2011) from the University of Ferrara (FC), Ferrara, Italy and by Don Orione Service s.r.l., Bergamo, Italy.

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European Journal of Inflammation

Official Journal of the Italian Society of Oral Pathology and Medicine

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