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Polyspecies biofilm formation involved in overdenture peri-implantitis

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LIGIA MUNTIANU[1*], IOANA SUCIU[1], CORINA CIRCEI [2], SORIN PENTA[1], ION VIOREL[3],CARMEN CURUTIU[4], IRINA GHEORGHE[4], ANCA MARIA RADUCANU[1]
1Dentistry Faculty Carol Davila, Bucharest, Romania
2Dentistry Faculty Titu Maiorescu, Bucharest, Romania
3Smile Focus Dental Cabinet, Bucharest, Romania
4 Faculty of Biology, University of Bucharest; ICUB, University of Bucharest, Romania
**Correspondent author: Ligia Muntianu, Tel 0736 476 484Email:* ligia.muntianu@gmail.com

Abstract

This report describes the investigation of potential causal factors involved in producing or promotion of peri-implantitis in a complex case with periodontal history, mixed prosthetic rehabilitation including maxillary implant overdenture and a partially edentate mandible. Biological harvests were sent to two different laboratories for cross checking the results. The results identified the presence of a polymicrobial biofilm with a mixture of species, predominantly anaerobic Gram negative rods, but some rare components of oral microbiota, as Streptococus thoralensis were also identified.

Keywords:peri-implantitis, implant overdenture, *Streptococcus thoralensis*

1. Introduction

Prosthetic rehabilitation of partial or complete edentulous patient using implants has updated to modern the prosthodontic techniques. Implants integrate by bone growth around implant body. At gingival level, there is never achieved a perfect seal as the epithelial junction in natural tooth. Thus, microorganisms could penetrate the tissue and in time could determine gingival retraction, bone resorption, peri-implantitis and finally implant loss (MADER & al. [1]). This happens more if personal hygiene is poor as in old people or children with brackets and orthodontic mini-implants (FERREIRA& al. [2]). Peri-implantitis represents in fact a progressive and irreversible disease of hard and soft tissues that surround implant and conduct in the end to bone resorption, decreased osseointegration, increased pocket formation and purulence (SMEETS& al. [3]). Dental plaque and biofilms develop in the mouth even if all teeth are lost (LUPPENS& al. [4]). The biofilm formation is a natural process and its composition could be related by inflammation of soft and hard tissue in different conditions of mouth health state (ADELL& al. [5], ALBRECKTSON &ISIDOR [6]). Prevention of biofilm development imply identifying the pathogen microorganisms involved in soft tissue inflammation and bone resorption. We analyzed a complex clinical case with maxillary overdentures on implants and partially edentulous in the mandible with periodontal disease history. We found a mixed pathogenic microbial species as we expected according studies reported in the literature (TORTAMANOA& al. [7], DE FREITAS & al.[8]) but we also found some interesting and rare bacteria that were not usually associated to oral microbiota.

2. Experimental procedure

First step: Beginning 2 months before starting the procedure of implantation, patient should not be going to have dental scaling. With 14 days before starting patient is informed

about the procedures and a consent was signed. A radiologic investigation is performed to evaluate the capability of implant to integrate, the bone and the soft tissue health. Patient should not use antibiotics or any medication. If there is such need procedure is postponed. Also, mouth wash should not be used 2 weeks before investigation.

Second step: (minimum 48h before microbial investigation): Patient came for dental check. He received a flyer with recommendations regarding the next 24h before investigation starts: overdenture should be used during night 24h before investigation, no tooth brushing, no drinking water, no food, in the morning of the investigation.

Step 3: (day of investigation) –clinical maneuvers were performed. Sample from different sites were collected: from the intaglio denture base, from the gingival sulcus of mandibular teeth and from the gingival sulcus at implant prosthetic connection level using sterilized bonding applicators as well as sample from gingival sulcus of implant after overdenture removal. From these samples, Gram staining smears were realized and inoculated in aerobic and anaerobic medium. Sampling was done as quickly as possible to avoid anaerobic germs exposure to aerobic conditions. The sample were transported at Clinical Microbiology Laboratory of the Hospital of Infectious and Tropical Diseases "Dr. Victor Babes", Bucharest, and Analysis Laboratory of the "Diagnostic Center of Cantacuzino Institute ", Bucharest, and both aerobe and anaerobe species were identified. Photos of dental field with overdenture in place, of sampling areas without overdenture in place, and of overdentures intaglio surfaces were realized.

3. Results

Implant-borne reconstructions are now more used then conventional fixed or removable partial dentures; an estimate number indicate that more than 2 million implants per year were made in the past decade. However, despite the frequently use, many problems associated with the implants are still unresolved, peri-implantitis has been identified in 28–56% of implant recipients and in 12–43% of their implant sites (MARUYAMA & al. [9]). One of the most important role in the failure of dental implants is attributed to bacterial infections. Peri-implantitis is a poly-microbial anaerobic infection (CHARALAMPAKIS & al. [10]). The most commonly found microorganisms in periodontitis and peri-implantitis, responsible for the failure of an implant are the Gram-negative anaerobes, like *Prevotella intermedia, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Bacterioides forsythus, Treponema denticola, Prevotella nigrescens, Peptostreptococcus micros,* and *Fusobacterium nucleatum* (PRATHAPACHANDRAN and SURESH, [11]). However, in contrast to periodontitis, peri-implantitis lesions harbor bacteria that are not part of the typical periodontopathic microbiota. In particular, *Staphylococcus aureus* appears to play a predominant role for the development of a peri-implantitis. This bacterium shows a high affinity to titanium and has according to the results of *Salvi & al.* a high positive (80%) and negative (90%) predictive value (SALVI& al. [12]). As another beneficial cause, smooth implant surfaces in comparison to rough surfaces can accelerate the peri-implant inflammation (SUBRAMANI & al. [13]). Immediately after implantation, implant surface is exposed to the oral cavity, and becomes covered by a protein layer – the salivary pellicle – and colonized by oral microorganisms, resulting a microbial biofilm. Biofilm development around implants occurs similar that in case of natural teeth, but the pellicle from dental implants has a more reduced capacity to absorb albumin, that means according to some authors a more reduce plaque formation around implants. Bacterial colonization occurs as early as 30 minutes after implant exposure in the oral cavity. After

pellicle is formed, first bacterial species attached to implant surface, followed by cell-to-cell adhesion of secondary colonizers. The peri mucosal seal of the soft tissue surrounding implant protects the base of the sulcus against chemical and bacterial substances. If the soft tissue is not seal, the peri-implant mucosa became from health to mucositis and possibly to peri-implantitis. In this case, a shift from Gram-positive facultative dominated microbiota to a Gram-negative anaerobic biofilm occurs. An unsuccessful implant is characterized by a greater proportion from certain species as *P. gingivalis, P. intermedia, F. nucleatum, T. denticola,* and *T. forsythia*, as well as *A. actinomycetemcomitans* and *Eikenella corrodens* and a lower proportion of the oral microbiota from a health person (LEE &WANG [14]).

The results of the processed biological samples demonstrated indeed the presence of a polymicrobial biofilm, according the most studies from literature regarding peri-implantitis, even if the prevalence of each strains was not the same. These differences were explicable, and probably due to patient's health state condition, presence or absence of teeth and their periodontal health state, saliva quality and so on. In figure 1 there are represented the bacterial strains isolated from every analyzed situs, and in figure 2 the microscopic aspects from some of them.

In our study, *Peptostreptococus spp*., an anaerobic, Gram positive, non-spore forming bacteria was present in all samples being possibly involved in promoting or producing peri-implantitis. In condition of immunosuppressed patients or traumatic conditions, it becomes pathogenic from commensal and it can cause soft tissue infections.

Veionella parvula is found normally in the gut and oral cavity of humans. In the oral cavity, the microorganism can form biofilms with other organisms with similar niches. The presence of *V. parvula* on the sample collected from the intaglio surface of the overdenture underlines dental plaque formation and accumulation on the internal aspect of the prosthetic device in a short span of time (one night), in conditions of good oral hygiene. As this microorganism has a mutual relationship with *Streptococus mitis/ oralis* which has also been found in analyzed samples shows the development of the biofilm on both prosthetic device and biological tissues in the oral cavity, as was expected. Its presence even if normally is considered nonpathogenic, it has been also linked to periodontal disease.

Prevotella melaninogenica, a strictly anaerobic, Gram-negative, non-spore forming coccobacilli was also found in this study. It produces a black pigment that can be easily seen in adults with rapidly progressing periodontitis lesions and usually grows in biofilm.

Peptococcusniger is a Gram-positive, non-motile anaerobic coccus was found in the biological sample harvest from de gingival sulci around implants.

Streptococcus thoraltensis is part of an unusual group of streptococci and was first described by Devriese et al in 1997. In our study, it was identified from gingival sulci around implants after overdenture removal. This bacterium is not specific for oral cavity in humans but for intestinal and vaginal microbiota of swine. Identifying it in dental plaque around implants after removing the overdenture is a rare thing and suggestive for complexity and diversity of the human oral microbiota (DHOTRE& al. [15]).

Other microorganisms were found in the biological collected samples, so we can state that peri- implantitis is a biological complication of implants in function.

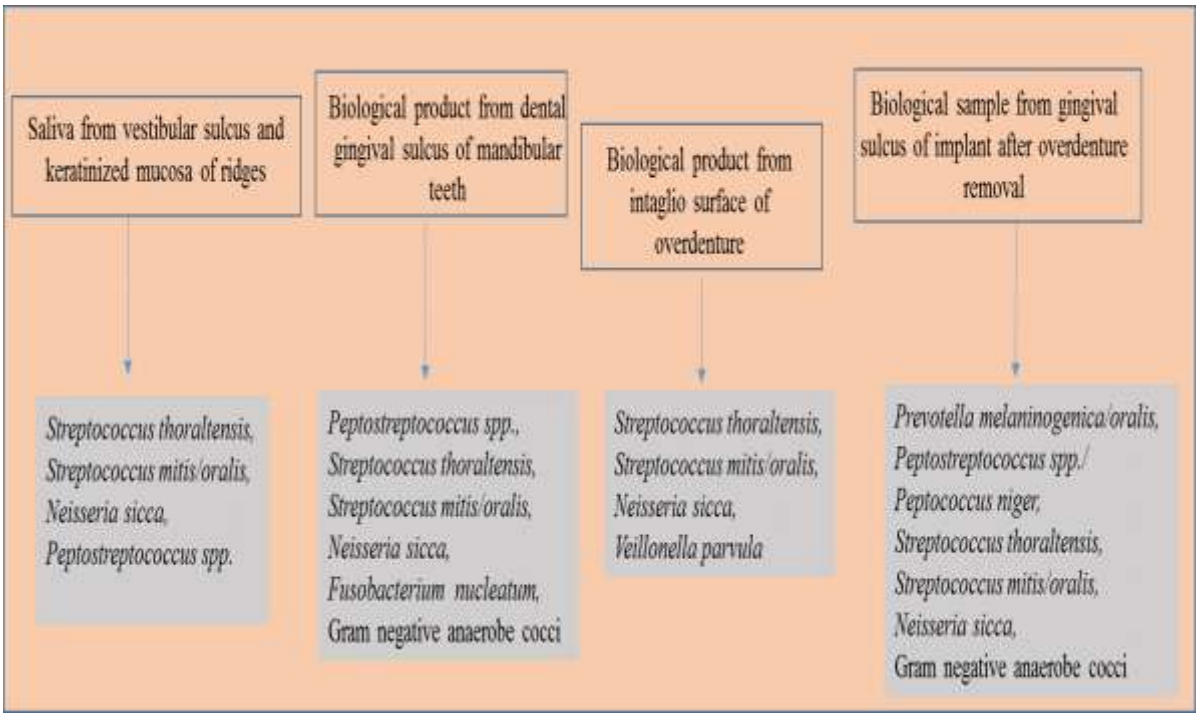

Fig. 1. The isolated microorganisms from every collected sample

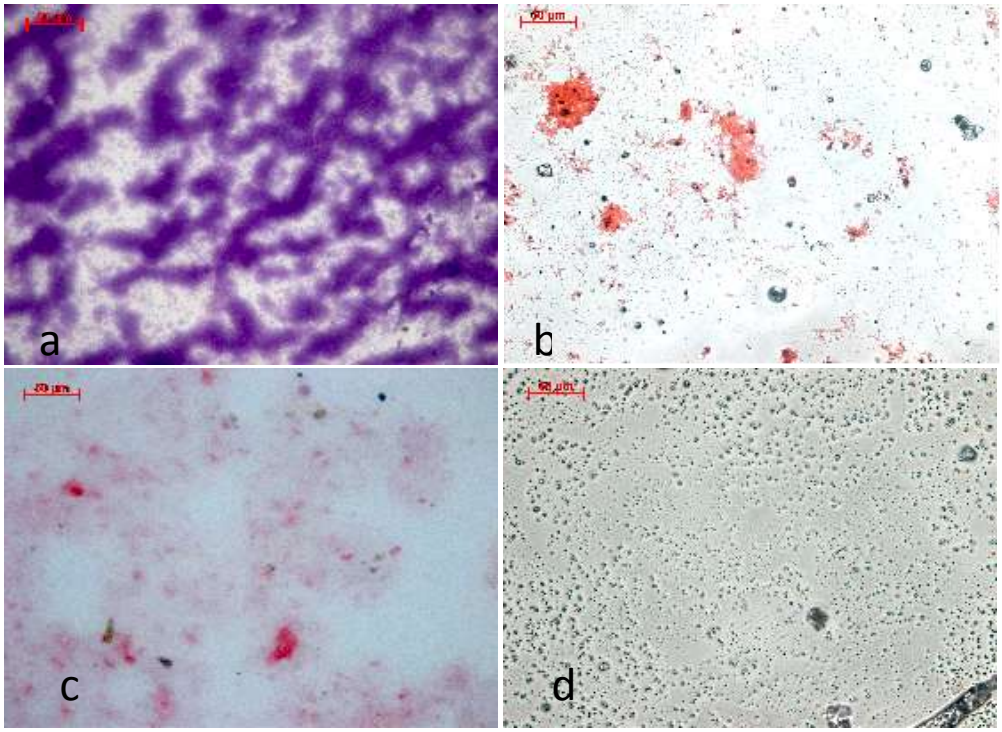

Fig. 2. Microscopy images of some of the isolated microorganisms: a- *Peptostreptococcus spp.*, b –*Veillonella parvula*, c – *Fusobacterium* sp., d- anaerobic Gram negative cocci (optic microscopy, 100X, Gram staining)

4. Conclusions

Oral biofilm-related diseases such as periodontal and peri-implant diseases are developed from the resident indigenous microbiota. As more implants are nowadays being placed, clinicians may encounter more complications. Implant complications have significant health and financial implications to both the patient and clinician. Therefore, understanding the etiology is warranted to establish adequate diagnosis and provide proper treatment. Our results showed that peri-implantitis is a polymicrobial infection with increased number of anaerobic and aerobic Gram negative bacilli. Osseointegration only doesn't ensure implant survival and peri-implantitis poses a threat on long-term survival.

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