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Traitement antiparasitaire des maladies parodontales et péri-implantites : suivi multicentrique à 12 mois

Article in *Actualités Odonto-Stomatologiques* · February 2013

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Abstract

For nearly a century, researchers have identified the protozoan parasite *Entamoeba gingivalis* from diseased gingival pocket. Recent use of PCR detected the parasite in 69% of diseased sites and demonstrated its absence in healthy gingival sites. The purpose of this cases report was to visualize this characteristic biofilm and evaluate periodontal results of dental clinics concerned with parasitic component of periodontal and peri-implantitis therapy.

KEYWORDS:

Parodontitis, amoebae, *Entamoeba*, biofilm, microscope, PCR, dental implant, peri-implantitis.

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Article available at <http://www.aos-journal.org> or <http://dx.doi.org/10.1051/aos/2013104>

IMPLANT

AOS 2013; 261: 22-28

DOI: 10.1051 / aos / 2013104

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Our knowledge of mouth parasites and their relationship to human disease are much less known than those of bacteria. The authors of the early century [1] relate that they find the amoeba *Amoeba dentalis* in gingival inflammation and found it looks like *Amoeba coli* of the human gut. Barret in 1914

announced specifically they found *Entamoeba gingivalis* in almost all cases of pyorrhea alveolar they examined. they speculate that the presence of this amoeba is of importance and was significant in periodontal disease. The beneficial results after the administration of emetine hydrochloride lead them to assign a pathological role to this organism; the following year, they extend review and confirm these observations over 300 cases. In their work, they assume continuously parasites live inside and at the expense of gingival tissue and the alveolar bone; they relate also that the amoeba is found in greater quantities at the apical end of the periodontal pocket. *E. gingivalis* in periodontal diseases is easily transmitted by droplets of infection and a fraction of saliva having this organism can be infectious [2]. It becomes clear this parasite can be transmitted from sharing cutlery and cosmetics as well as by direct contact. Kofoid [1] quotes Hinshaw and Sinonton which concluded by saying that the amoeba was never found in a strictly normal mouth and invariably found in a typical periodontitis from its inception to its end. The existence and deepening of gingival pocket accompany invariably this type of inflammation. In addition, patients who take good care of their mouth seem equally likely to have the amoebic infection. Increasing with age, the incidence of parasitic infection progresses rapidly until that beyond the age of 40 would reach 75% of the population. Subsequent studies are rather descriptive. Another level of sensitivity and specificity was achieved by using reaction polymerase chain reaction (PCR) method. First, the identification of *E. gingivalis* PCR [3] failed to demonstrate clearly the presence of amoebae in patients with only 6% positive. Lack of sensitivity of the primer pair was demonstrated by Trim et al. [4], who used a method of DNA purification most appropriate, up to 27% of positive samples in patients with the same primer, while 69% were obtained with a set of primer pair and real-time PCR which is more accurate. It seems important to note that *E. gingivalis* was not detected in any healthy mouth or in any healthy tooth of patients affected by periodontal disease.

A Canadian dentist, Dr. Trevor Lyons [5-8], monitoring by microscopic observation the crevicular biofilm sulcus of its patient for more than 10 years, says evidence of invasion of the gingival periodontal sulcus by *E. gingivalis*. Its

mode of diagnosis and treatment is based on medical diagnostic and therapeutic approach just as cousin intestinal parasite *E. histolytica*. This clinician also reports that oral parasites are found in all cases of periodontitis, they nourished from white cell nucleus as well as red cell and a substantial clinical improvement is invariably obtained by their elimination. Since amoeba, when present, are found constantly at the base of the infected pocket, samples are collected primarily in these specific area. Oral parasites are found only in diseased sites. He concludes that despite the adage *E. gingivalis* be a commensal, evidence demonstrates that this is an aggressive pathogen. It therefore appears, as described in his book, [8] that the vast majority of patients affected by periodontal disease are infected with oral parasites and that removal of these protozoa is followed by the arrest of the disease and its resolution, including bone regeneration. Using the knowledge of Lyons, and our clinical experience for 26 years in the periodontal microscopy, hygiene only shows it is not sufficient to get rid of the mouth protozoa in most cases of periodontitis. In a study by Linke [9] conducted in 10 patients with advanced periodontitis, all subjects were infected by *E. gingivalis*. The recommendation he poses to obviously take multiple sites in the same patient to avoid false negatives. His conclusion is that the data reported generally in the literature on the incidence of *E. gingivalis* after one patient sample is too low and should be interpreted with caution. *E. gingivalis* is present in 65.4% of subjects 30 to 34 years [10]. The prevalence of the protozoa is much more important in case of periodontal destructive disease compared to subjects apparently unharmed. It is therefore concluded, in 1990, there is a correlation between diseased periodontal tissue and protozoa infestation just like in 1929 as a result of the significant Kofoid study [1]. Like other amoebas, *E. gingivalis* has a surface protein related to fibronectin [4]. This occurs in the mechanical adhesion and phagocytosis, which is involve the in the early stages of periodontal tissue destruction. Even more important, various forms of amoebae are commonly found in tap water and tubing in dental units [11] and some of these protozoa are considered pathogen.

METHODS AND RESULTS

Data for this study came from our files and the clinical retrospective study of 5 dental clinics concerned by the parasitic component of periodontal care. We therefore conducted therapies according to the recommendations of Lyons, taking routine observation of fresh smears

crevicular biofilm, taken from deepest periodontal pocket affected. We observe deep crevicular plaque specimen on phase contrast microscope obligatory mounted on patient saliva to reduce protozoa deformation. This allows to quickly find all patients with the amoeba *E. gingivalis* achieved some degree of periodontal disease (Fig. 1).

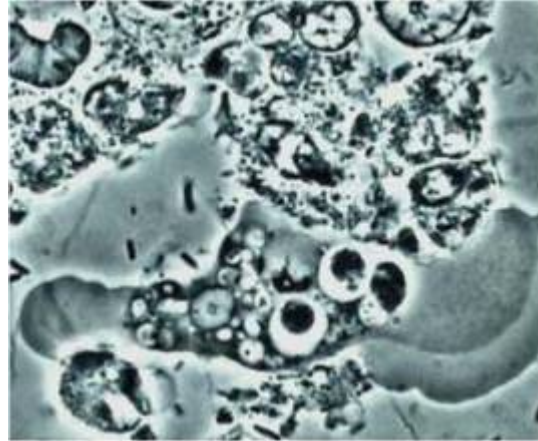


Fig. 1: (Magnification 1000x) Typical amoeba surrounded by neutrophils, bacilli and spirillae. *E. gingivalis* is easily distinguished by its rounded nucleus to the left, central karyosome and chromatin at periphery of the nucleus. One well-formed pseudopod is visible on the left and a lamellar pseudopod cytoplasm at periphery on the right. Three vacuoles recall recent ingestion of nuclei from leukocytes.

Patients in complete periodontal health without gingival bleeding do not exhibit parasites but present mainly non-mobile coccoid and filamentous bacteria forms in (Fig. 2) in agreement with the work of Sockransky [12], Nisengard and Newman [13].

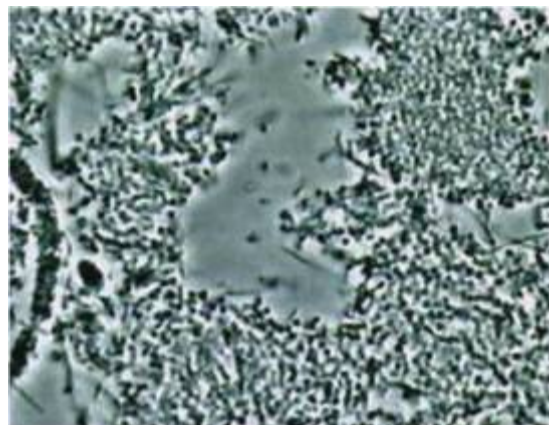


Fig. 2: Normal flora in a healthy periodontal patient. Non-motile bacteria coccoid form are present, accompanied by various lengths filaments. On the left a long filament surrounded by coccoid reminds of *Corynebacterium matruchotii*. Note the absence of inflammatory cells.

The invasive and locomotion capacity of amoeba are important aunts. We currently observe that

the amoeba is often motile, emitting a pseudopod at its anterior part and presents the formation of an uroïde in its distal portion, phenomenon known as "capping" [14] (Fig. 3).

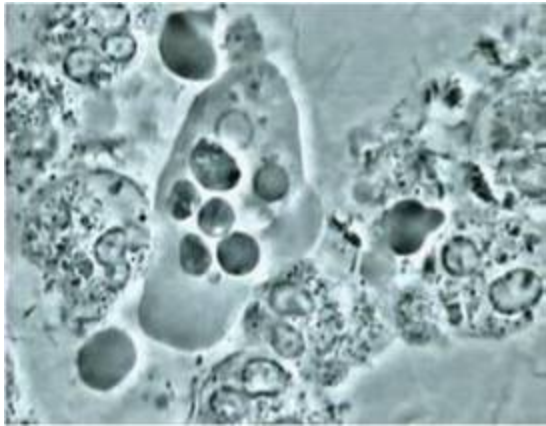


Fig. 3:
A rather lively amoeba accompanied by motile bacteria converges to a cell granulocyte. Digestive vacuoles composed of PMN nuclei phagocytosis are of good dimensions and clearly visible. The distal portion of the amoeba is consistent with the uroid phenomenon called "capping".

In the human pathogen *Entamoeba histolytica*, the uroïd seems to play an important role in the escape of the immune response from the host. Adhesion, inquilinism (fig. 4) and phagocytosis (Fig. 5), another important factors related to the pathogenicity of the species *E. histolytica*, are easily observable.



Fig. 4:
E. gingivalis in intimate contact with large bacterial filaments forming palisade indicating strong affinity in a case of pregnancy-related periodontitis. The notion of organization and inquilinism leaves advanced ergonomics suspicion in these parasites.

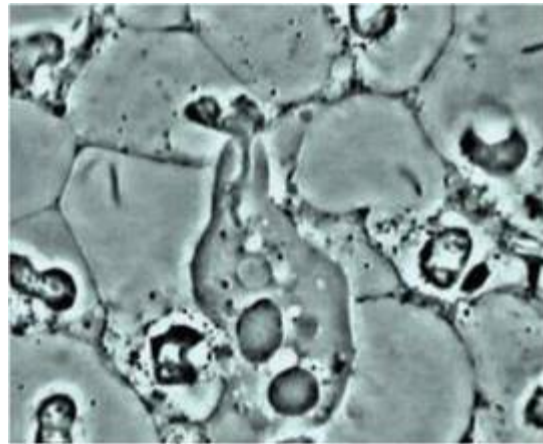


Fig. 5:
Beginning of leukocyte phagocytosis process by an amoeba in active periodontitis patient. Note the intra cellular denser cytoplasmic nucleus from granulocyte which is gradually swallowed. In periphery, ghosts carcasses of denucleated white cells, releasing out of control enzyme that will dissipate on surrounding tissues.

The amoeba leaves behind, after PMN phagocytosis, a denucleated neutrophil recessed cell filled with proteolytic enzymes also described as a ghost cell, rendering normal apoptosis impossible to occur [15]. Removal of inside part and more specifically their nucleus, surely prevents the formation of "NETs" (Neutrophil Extracellular Traps) organization of chromatin fibers (organized nuclear structural DNA and protein) exposed out of the cell and on which are fixed enzymes, normally constituting lytic killing traps for pathogens [16]. This phagocytosis of the nucleus by the amoeba (that we called "exonucléophagy") could be a cause of the inability of the immune system to resolve the infection during periodontitis or peri-implantitis, leading to a chronic inflammatory dysregulation. The presence of the amoeba is not trivial in the periodontal sulcus of patients (Fig. 6) and can present a high degree of multiplication and the formation of reel amoebic nests (fig. 7).

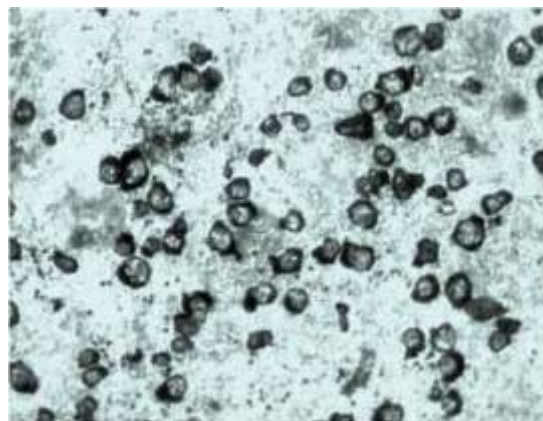


Fig. 6:
(Magnification 100x) Presence of about fifty amoebae on a dark field preparation. The darker surrounding portions appears as

pseudopod while the lighter inside part concentrates digestive portion of vacuolar endoplasm.

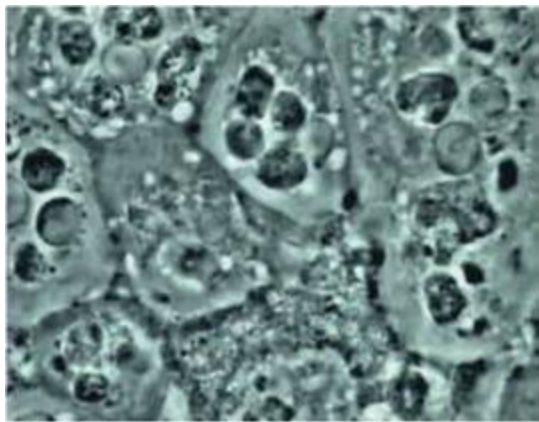


Fig. 7: Clusters of parasites in a case of rapidly progressive periodontitis suggesting a major nesting of *E. gingivalis*. This strongly reminds of amoebic liver abscess and surrounding tissue destruction. Six amoeba trophozoites bathe in purulent substance.

As proposed by Lyons, the used therapy mainly target the elimination of the amoeba from the infected pocket. We therefore conducted monthly checkups treating with local therapy using 1% hydrogenated water, baking soda and salt powder as dentifrice and used metronidazole cream 10% applied topically for a period of few months and finally completed lastly by systemic antiparasitic medication to permanently eliminate amebiasis if persistent. Invariably, clinical signs of healing periodontitis appears and infectious biofilm fades away, giving way to a free neutrophils and parasites normal non mobile bacterial flora. Once the pathogenic flora is removed, bleeding disappears, retraction of the gum quickly happens, then the more easily observable residual calculus is finally removed. We obtained in 2003 [17] in a group of 20 new patients with advanced and aggressive periodontitis therapy having completed this phase, the elimination of 94% of periodontal pockets exceeding a 3 millimeter considered normal sulcus calculated according to format specification proposed by Harrell and Nunn [18] at one year.

Initial biofilm mainly composed of parasites, neutrophils, spirochetes and motile bacteria is replaced by a free white blood cells biofilm, consisting of motionless coccoid bacteria and filaments as in all cases of periodontal health. Healing is stable over time and reinfection factors are controlled following the therapy that is entourage, spouses, close family, animals

companion and traveling in high tropical risk areas just as indirect transmission vectors, in any parasitic disease of the same nature in medicine.

In 2011 we completed a retrospective study of five French clinics using the Bonner-Lyons-Dunoyé antiparasitic protocol. Clinical records for 632 chronic and aggressive periodontitis patients, on a based survey charting in 6 points per tooth, where the normal is considered 3 mm or less, we found after antiparasitic periodontal treatment average sulcus clinical pockets cicatrisation equivalent to 95.7% for all patients at 12 months.

In the same vein, 32 peri-implantitis patients were evaluated for biofilm microscopy with a fresh smear. For example, typical X-ray image (Fig. 8) and clinical (Fig. 9) of the implant in position # 23 during therapy done by the patient shows vertical bone loss approaching the apex of the implant.

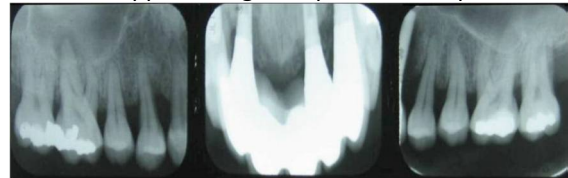


Fig. 8: Radiograph case of peri-implantitis with visible loss of vertical bone in implant situation No. 23.



Fig. 9: Same patient in pretreatment periodontal therapy Originally treated by placing implants in a previous dental clinic. Periodontitis situation not solved for 15 years.

Pressure on the gum evacuate purulent yellowish biofilm typical of periodontal abscess.

At microscopy, the image of the smear examination shows (fig. 10 and 11) exactly the same biofilm as chronic or aggressive periodontitis with amoebae and white blood polymorphonuclear apoptotic neutrophil activity.

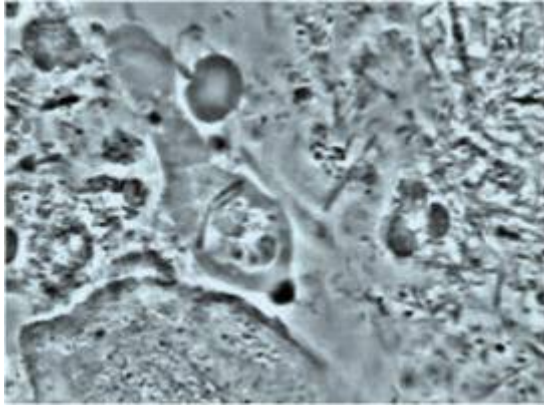


Fig. 10:
Biofilm in this case with peri-implantitis, presence of polymorphonuclear neutrophils and unipolar amoeba with an uroide at its distal end moving up the image.

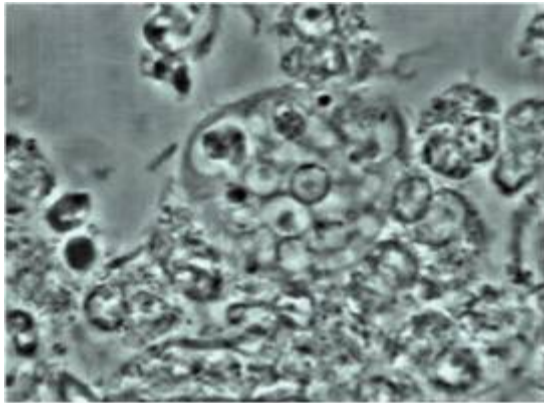


Fig. 11:
Same case of peri-implantitis with an amoeba with its characteristic centered nucleus, it's formed phagocytized vacuoles cores and surrounded by PMN.

DISCUSSION

Researchers using phase contrast microscope identified the protozoan parasite *E. gingivalis* from periodontal infected pockets for almost 100 years. Recent studies have developed a molecular biology approach to determine the presence of *E. gingivalis* in periodontal disease pockets and gingival healthy sites. For this, a conventional PCR [3] was used with paper points inserted in healthy periodontal pockets or periodontal disease pockets. More recently, Trim et al. [4] have improved PCR probes and obtained similar results as microscopic data. This latter technique is adding a new appropriate tool to assess the presence of *E. gingivalis*.

Protozoan was not detected in any of healthy gum sites nor with any of the different PCR technique. This new methodology allows us to add a new eukaryotic marker status of the gingival pocket.

Moreover, no amoeba were detected in a healthy pocket of a patient diagnosed as having a localized periodontal disease. One relates the possibility *E. gingivalis* is able to develop proteolytic enzymes which contribute to the pathogenesis of periodontitis. The results of this last investigation gives a rational basis to help understand the potential etiologic role of these parasites. Although periodontal disease still holds the attention of medical and dental researchers as well as local factors, systemic factors, habits such as smoker, have been implicated in the process of periodontal disease, the success has often been disappointing, with an annual bone loss of 0.15 mm [18]. Today, the predominant treatment modality is mechanical in nature and promotes the removal of a portion of the oral tissues. Without the aid of microbial surveys, the initial result appears favorable; however, disappointed often is inevitable. More paradoxical regarding implantation techniques, we observed the presence of these parasites accompanied by the polymorphonuclear neutrophil at microscopy in the clinical situation of bone loss and long-term faulty integration implant. In one particular dental clinic specifically involved in peri-implantitis therapy, patient's microscopic evaluation allowed us to visualize the presence of the amoeba in 31 patients out of a total of 32. The periodontal therapy case of peri-implantitis presented after treatment decrease the depth of periodontal pockets on natural teeth except on the implant that was affected of successive parasitic infection and because of mobility at 6 years.

The most common denominator in all chronic or aggressive periodontitis, and peri-implantitis remains for those who want to observe the presence of oral parasites. Our clinical experience shows that their careful and complete removal brings on natural teeth a rapid and painless healing, reliable over time and permit to complete residual subgingival detartrage with ease (Table I).

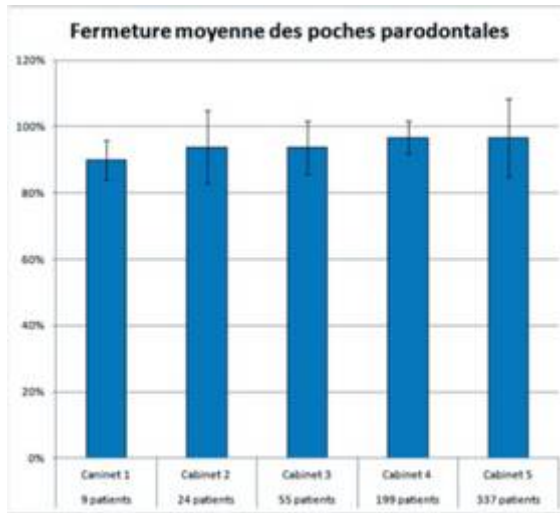


Table I:
5 dental clinics of different types: general practice (row 1, 2, 3), and dedicated periodontal practices (row 4, 5) results showing closed periodontal pockets after the establishment of Antiparasitic Control Protocol. Results are essentially similar with high degree of periodontal healing whatever goodwill.

The re-attachment of the periodontal ligament in a way similar to any healing of the human body and angular bone defects can fill over years the same way (Fig. 12 and 13).



Fig. 12:
Pretreatment radiograph with mobility I of tooth No. 11. Note the angular defect of the distal this tooth.



Fig. 13:
Post treatment radiograph followed at ten years after porcelain. The tooth No. 11 formerly mobile and promoted to mechanical conventional therapy and extraction is still present. Instead, functional not mobile post therapy trabecular bone have virtually filled the distal vertical defect.

Tooth initially dedicated to extraction, remains without bone graft and implant. Disposal parasites in cases of peri-implantitis seems rather more difficult or even impossible in view of the presence of a persistent inflammation [19] consisting of PMN providing a constant supply of food to the trophozoites through the exonucleophagy process. All this seem to confirm the ability of *E. gingivalis* to invade and destroy gum tissue inflammatory cell first fragilised by bacterial gingivitis. Remind that with our colleague's parasitologist, destruction of liver tissue is caused by the remnants of neutrophils after phagocytosis. According to Orozco [20] and Akuffo [15], the degeneration and cell lysis cause the release of proteases, such as phospholipids, collagenase and cysteine protease capable of degrading the laminin, fibronectin and collagen. Note *E. gingivalis* frequently may contain between 3 and 5 phagocytized nucleus but this can also easily exceed 10 nucleus (fig. 14).

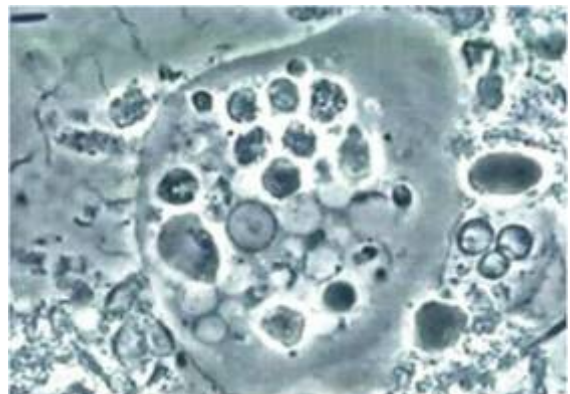


Fig. 14:
Amoeba present in case of an aggressive periodontitis. Note following PMN phagocytosis of nuclei more than ten mass being digested in amoeba endoplasm. The trophozoite nucleus with its distinctive ring with central karyosome and chromatin at periphery.

One amoeba can also phagocytize up to 4 nuclei of neutrophils by four different pseudopodia at the same time (Fig. 15).

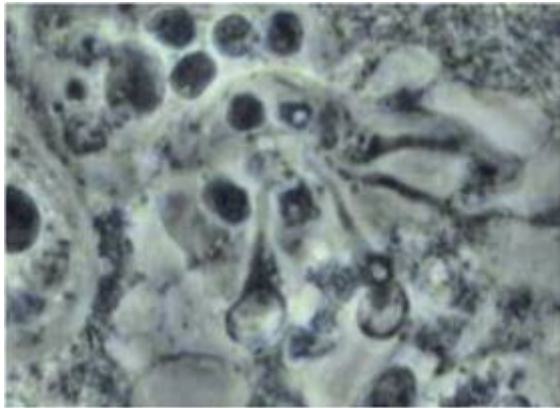


Fig. 15:
Large amoeba in full activity of simultaneous phagocytosis, devouring 4 neutrophils nuclei and leaving at the periphery white denuded cell logically out of control.

In contrast, a patient with a biofilm consisting of a perfect commensal flora (coccioid bacteria motionless filaments and absence of neutrophils) will no doubt in this field increase his chances of success with bone grafting preparation and dental implants in a context where it cannot become infected by means of direct self-intraoral contamination. (Fig. 16-19).



Fig. 16:
Pretreatment radiograph procedure posterior sextants for autogenous graft apposition and fixed prostheses implants in a situation of commensal biofilm.



Fig. 17:
Laying bilateral grafts from posterior mandibular access.



Fig. 18:
Implants and fixed prostheses in place at 10 years.



Fig. 19:
Clinical situation in microscopic biannual control, good maintenance of a microbial commensal flora.

From a parasitic view, the latter criterion essentially required for periodontal healing remains prevention of reinfection by eliminating transporting vectors and more importantly periodontal pre-implant situation, giving assurance with good oral habits and monitoring of the family environment as stipulated in the normal medical field of parasitic infections.

CONCLUSION

Current observations confirm the amoeba *E. gingivalis* plays a very specific role in the pathogenesis of periodontitis and peri-implantitis. It is reasonable to assume that *E. gingivalis* can be the agent that allows the passage of a benign

disease, gingivitis, into a destructive disease, periodontitis or peri-implantitis. Moreover, the typical parasitic pathogenicity factors which are high mobility and capacity of phagocytosis lead one to believe that *E. gingivalis* trophozoite share with his close cousin the virulence traits of *E. histolytica*, modulation of the host response lysing the host cells and surrounding tissues. The amoeba *E. gingivalis* clearly seems to handle this response by inactivating immune factors (by mean of denucleated neutrophils) that otherwise would protect from the jaw bone destruction. Its presence in the biofilm is a high constant in periodontal and implant disease. *E. gingivalis* also has been identified as responsible for pulmonary abscess [21] and recently found in mandibular osteomyelitis case [22]. Its elimination, concomitant with anaerobic bacteria, ensures through easy microscopic targeting produce effective healing of chronic and aggressive periodontitis. Comparative studies of other species colonize humans could help to understand issues concerning fundamental biology virulence, host-parasite interactions and public health concerns facing the periodontal and implant rejection disease. Sequencing genome of this species is promoted, and hope be a first step to understand its biology and its interactions with the human host [23]. Many clinical and biomolecular aspects similar to *E. histolytica*, responsible for amoebic dysentery [24, 25] should merit the attention of researchers typically regarding pathogenic genes characters. Future studies are encouraged to determine the genome of this parasite and better establish its biomolecular parameters compared with virulent specie *histolytica*, *dispar* and *Rahman* strain [23].

E. gingivalis act in various forms of periodontitis including peri-implantitis as an intrusive pathogen in light of his blood-sucking activity and degradation of cellular immunity of the host it generates. Its detection in periodontal sulcus should encourage clinician to systematically propose antiparasitic therapy similar to equivalent amoebae medical disease thus ensuring a better chance of success, promoting a return to the commensal flora, guaranteeing periodontal health and dental implant osteointegration in a more favorable environment.

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ACKNOWLEDGMENTS

Special thanks to Julien Santi-Rocca, PhD in biochimie et biologie moléculaire, spécialisé en parasitologie de l'université Pierre et Marie Curie, Paris VI, for his contribution and his recommendation during this study. Also thanks to Mme Solange Dunoyé, communicatrice et directrice de l'Institut International de Parodontie for her contribution to communication during the study.