ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The role of light microscope and classic culture in detection of periodontal pathogens, Review of the Literature

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ABSTRACT

More than 700 bacterial species are able to colonize the subgingival periodontal pocket of humans. Periodontitis is a multi-factorial chronic inflammatory disease of the periodontium with varying degrees of bone loss. Destruction of periodontium starts by colonization of gram-negative anaerobic microorganisms. Several methods have been used for identifying the involved species. The aim of this review is to identify the most virulent periodontal pathogens at the classic cultured medium.

Key words: Microscope, Classic culture, Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola.

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ISSN: 0975-8585

INTRODUCTION

Although the human fetus is sterile, after birth several microorganisms start to colonize the whole body. After 2 years the entire human microbiota counts approximately 10^{14} microbial cells. On the other hand our body contains *10 times more bacteria* than human cells which comprises 2 kg of the total body and amazingly heavier than the brain's weight. The colonization of the oral cavity starts after birth by mainly facultative and aerobic bacteria. The first colonizers of the oral cavity include *Staphylococcus* spp, *Streptococcus* spp, *Lactobacillus* spp, *Actinomyces* spp, *Veillonella* spp and *Neisseria* spp. After tooth eruption, a more complex oral microbiota can be colonized at the teeth surfaces. Recent studies have revealed that about 500 different bacterial species have the ability to colonize the mouth and any individual may have around 50 to 150 different species [1, 2].

Discovery of dental plaque dates back to the seventeenth century, when Anton Von Leeuwenhoek, who invented the Microscope, saw microbial aggregates on the scraping materials of teeth surface [3, 4].

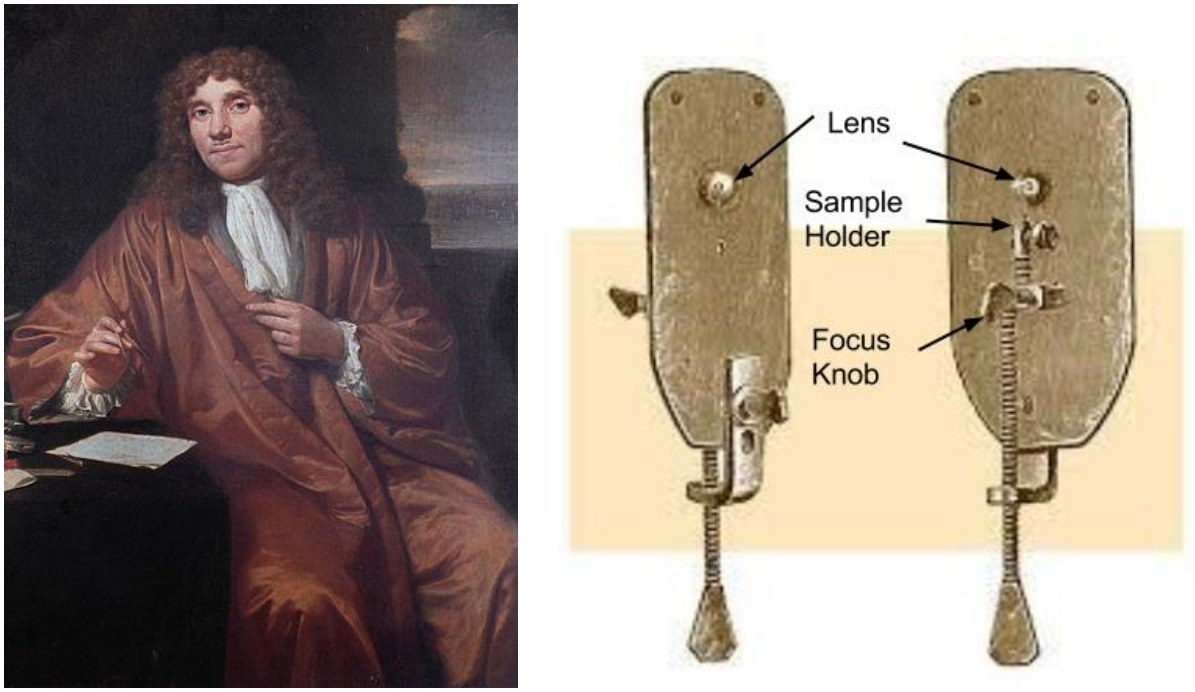

Fig 1. Antonie van Leeuwenhoek who invented the first microscopes for recognition of dental plaque bacteria and his microscope

Dental plaque is a structurally and functionally organized biofilm that form through several gradual stages. The first stage includes the formation of acquired dental pellicle, followed by the adhesion of first bacterial colonizers to the tooth surface. In this stage, the predominant teeth colonizers comprise mainly *Actinomyces* species and *Streptococcus* species, in particular *Actinomyces israelii, Actinomyces naeslundii, Actinomyces oris, Streptococcus mitis, Streptococcus oralis, Streptococcus sanguinis, Streptococcus intermedius and Streptococcus gordonii*. In the next stage secondary bacterial colonizers attach to the first colonizers using their specific surface molecules known as adhesins and receptors. This process is also called "Co-adhesion" or "Co-aggregation" which is necessary for the bacterial multiplication and synthesis of intermicrobial exopolymer matrix to form a mature biofilm. Secondary colonizers include mainly *Campylobacter gracilis Campylobacter rectus, Campylobacter showae, Eubacterium nodatum, Aggregatibacter actinomycetemcomitans* serotype b, *Fusobacterium nucleatum* spp *nucleatum,Fusobacterium nucleatum* spp *vincentii, Fusobacterium nucleatum* spp *polymorphum, Fusobacterium periodonticum, Parvimonas micra, Prevotella intermedia, Prevotella loescheii, Prevotella nigrescens, Streptococcus constellatus, Tannerella forsythia, Porphyromonas gingivalis* and *Treponema denticola* [5-9].

ISSN: 0975-8585

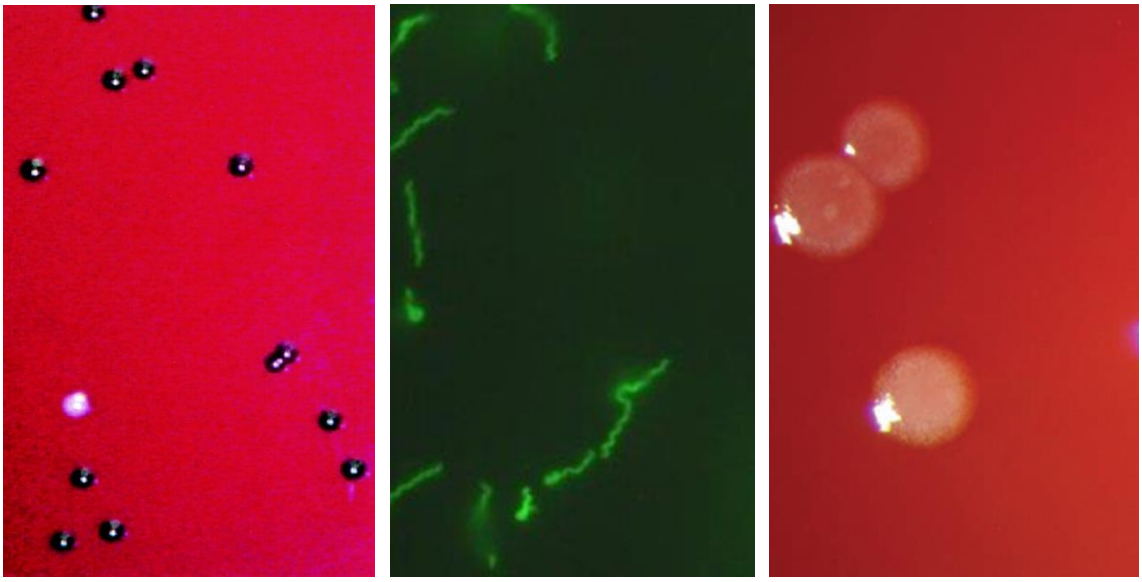

Fig 2. Red complex at the cultured medium. The left picture includes *Porphyromonas gingivalis microcolonies,* the middle *Tannerella forsythia and* the right picture *Spirochetes.*

Porphyromonas gingivalis

Porphyromonas gingivalis is an important periodontal pathogen that has been strongly investigated for its specific characteristics. This bacteris is a black-pigmented Gram-negative, anaerobic, non-motile, asaccharolytic rod with coccal to short rod appearance at the solid culture [Oliver & Wherry 1921] and has been intensely associated with severe forms of periodontal disease [10-13]. *Porphuromonas gingivalis* produce collagenase, several proteases for destroying immunoglobulins called "gingipain", hemolysins, hydrogen sulfide, fatty acids, endotoxin, ammonia, indole and etc [14-16].

Tannerella forsythia

Tannerella forsythia is a Gram-negative, anaerobic, spindle-shaped, highly pleomorphic rod that was first described in 1979 [17]. Growth of this species in the culture media is difficult and usually requires 7-14 days for small colonies to develop. *Tannerella forsythia* is associated more likely with gingivitis, chronic and aggressive periodontitis [18-21]. It has been reported that overweight or obese individuals have an overgrowth of *T. forsythia* compared to normal weight individuals and have a higher risk of developing periodontal disease [22]. *Tannerella forsythia* requires N-acetyl muramic acid for the growth which can be provided by co-cultivation with F. *nucleaium* in the medium culture [23]. Virulence determinants of this species include Surface associated glycoproteins (S-layer, TfsA and TfsB) for attachment to the epithelial cells, BspA surface protein for adherence and invasion and interaction with the innate host response via TLR_2 and TLR_3, Sialidases (SiaHI) for degradation of host oligosaccharides and Protease (PrtH) for epithelial barrier disturbance [24-26].

Spirochetes

Spirochetes are Gram-negative, anaerobic and highly motile bacteria that are visualized as helical-shaped colonies in the strict anaerobic conditions and a specific medium culture. Several studies have demonstrated the role of this microorganism in the etiology of destructive periodontal diseases. The most important subspecies of this group includes *Treponema denticola, Treponema vinccentii and Treponema Socranski* and *Treponema pallidum.* The numbers of this group interestingly increase in sites with increased pocket depth. Of this species *Treponema denticola* has gained more interest because of membership in red complex which is the most destructive periodontal pathogenic complex [27-29] and having several virulent factors including: Major sheath protein (Msp) which is a cell surface protein associated with adherence, Leucine rich protein (Lrr) for bacterial and epithelial cell adherence, Dentilisin (PrtP) for degradation of host cell matrix proteins and Trypsin-like protease (OpdB) for Protein and peptide degradation [30, 31].

CONCLUSION

Periodontitis is a prevalent and progressive disease that affects individuals with poor oral hygiene and lead to the loosening and finally loss of teeth. Other etiologic factors may include environmental conditions, genetic polymorphism, nutritional status and the psychological stresses that can affect disease development and progression.The destruction of periodontitis is believed to result from a mixed bacterial infection of the periodontal structure, and several clinical studies have demonstraded the role of *Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola* in the pathogenesis of the disease. For this reason identifying specific pathogens via laboratory experimental approaches of the cultured medium and several physical or chemical anti-plaque strategies have been applied [32-37].

ACKNOWLEDGEMENT

I take this opportunity to appreciate all of the Mashhad Department of Periodontology and Implant dentistry faculty members for their help and support. The alphabetical list of professors includes Hamid Reza Arab, Seyed Ali Banihashemrad, Kazem Fatemi, Habib allah Ghanbari, Amir Moien Taghavi, Majid Reza Mokhtari, Mehrdad Radvar, Mohammad Ebrahim Rahmani, Naser Sargolzaei and Mahmoud Tamizi. I thank all of them for the constant encouragement, support and attention.

Declarations of Interests

The authors confirm that this article content has no conflict of interest.

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