Diseases Associated with Oral Polymicrobial Biofilms

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Abstract: The human body can be defined as a symbiotic interaction between eukaryotic cells and prokaryotic cells. The body contains approximately ten times more microbial cells than mammalian cells. Fortunately, a large segment of the microbiome is both helpful and non-harmful, and constitutes the normal microbiome throughout the entire body. The digestive tract and the skin are the most microbial-rich sites containing approximately 1000 species of bacteria. The mouth is another site harbouring a diverse, abundant, and complex microbial community. Microorganisms in the mouth accumulate on both the hard and soft oral tissues and are frequently organised as microbial biofilm. This biofilm is usually harmless, yet under certain conditions, it may locally and systemically be the source of infection. This review focuses on oral biofilm formation and the diseases that may cause.

Keywords: Biofilm, dental plaque, caries, candidiasis, periodontal diseases.

BIOFILM FORMATION

The human body is host to a significant number of microorganisms [1, 2]. These microorganisms can be planktonic or free-living, and frequently organise themselves into a consortium of microbes called biofilm that adheres to a favourable support, interacts, and produces extracellular matrix [3]. Different steps ensue, leading to the formation and maturation of the biofilm [4, 5]. Microorganisms initially attach to available surfaces, after which time planktonic microorganisms undergo phenotypic change with significant attachment (adsorption) through a thin extracellular polymeric material (Fig. **1**). Following this irreversible attachment, the microorganisms in the biofilm proliferate and optimally interact to ensure their sustainability in the imposed environment. Following the proliferative phase is the production and deposition of thick extracellular matrix (mature biofilm) which procures chemical as well as physical protection for the microorganisms. The formed biofilm then undergoes focal dispersion through specific signals leading to microbial release (Fig. **1**). Free microbes are then able to spread to other locations to form new biofilm [4-7].

Microbial biofilm has an important side effect on human health (Fig. **2**). Indeed, over 60% of infections encountered in humans are caused by biofilms [8]. The most accessible biofilm is found in the oral cavity and is called dental plaque [9, 10] which accumulates on hard and soft oral tissues and also on restorative dental materials (Fig. **3**). Dental plaque/biofilm is involved in various dental diseases such as dental caries, oral candidiasis and periodontal disease [11-13].

ETIOLOGY OF DENTAL CARIES

Dental caries is characterised by localised destruction of the tooth following long contact/interaction with acidic products that result from the bacterial fermentation of dietary carbohydrates [14]. Dental caries is a chronic disease that progresses slowly in most individuals [15]. Caries can be found in both the crown and root portions of both primary and permanent teeth, as well as on smooth, pitted, and fissured surfaces. Early caries signs are very difficult to detect with traditional clinical and radiological procedures [16]. Dental caries is promoted by endogenous factors, with a primary role attributed to the dental plaque/biofilm in conjunction with salivary flow and composition. Several exogenous factors affect caries development, including exposure to fluoride, dietary sugars, and dental hygiene [17, 18].

Humans are affected by caries at different ages. For instance, young children (12–30 months) have a specific caries pattern that differs from that in older children. In this age group, caries attacks the maxillary primary incisors and first primary molars [19,20]. Compared to the mandibular incisors, the upper incisors are highly vulnerable due to their reduced contact with the tongue and the saliva [19, 20].

Caries is the primary source of tooth loss in children [21] and results from an ecological imbalance in the physiological equilibrium between tooth minerals and dental plaque/biofilm [22, 23]. Teeth provide non-shedding surfaces that are favourable to oral microbial adhesion, colonisation, and biofilm formation [24]. Oral bacteria, primarily *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus spp*, are major constituents of dental plaque/biofilm responsible for caries diseases [25, 26]. *S. mutans* adheres strongly and releases acids by carbohydrate fermentation, which demineralises the tooth and results in cavitation within the tooth [27]. If not prevented, this cavitation provides an ecological niche where micoorganisms gradually adapt to the reduced pH [28]. Formation of a cavitated lesion protects the biofilm, thus enabling the caries to progress [29]. Dental caries can be detected in the enamel, as white spot lesions, referring to tooth surface demineralisation beneath the biofilm, and can also attack the tooth root, leading to its softening. This ultimately facilitates bacterial penetration and damage

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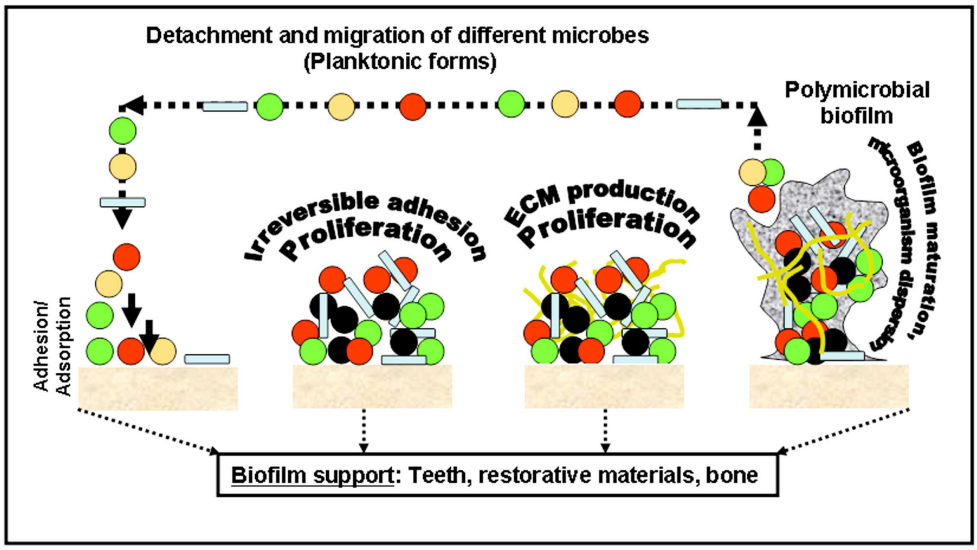

Fig. (1). The different stages of polymicrobial biofilm formation in the oral cavity.

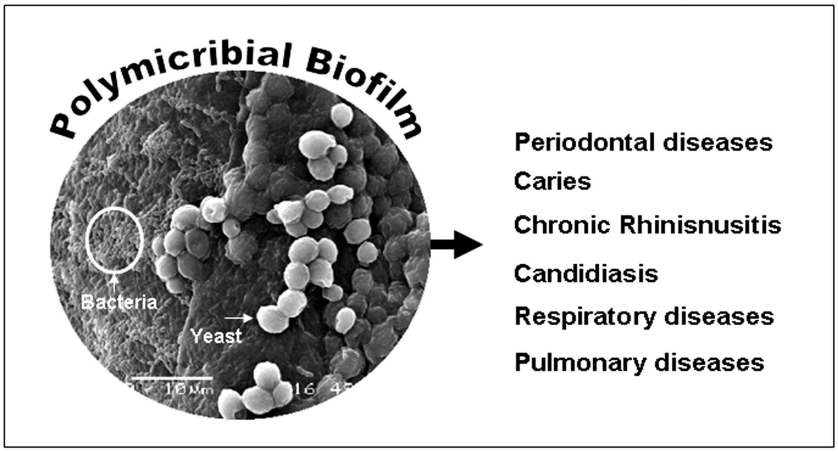

Fig. (2). Effect of dental plaque/biofilm on human health. Biofilms may lead to different local and systemic pathologies.

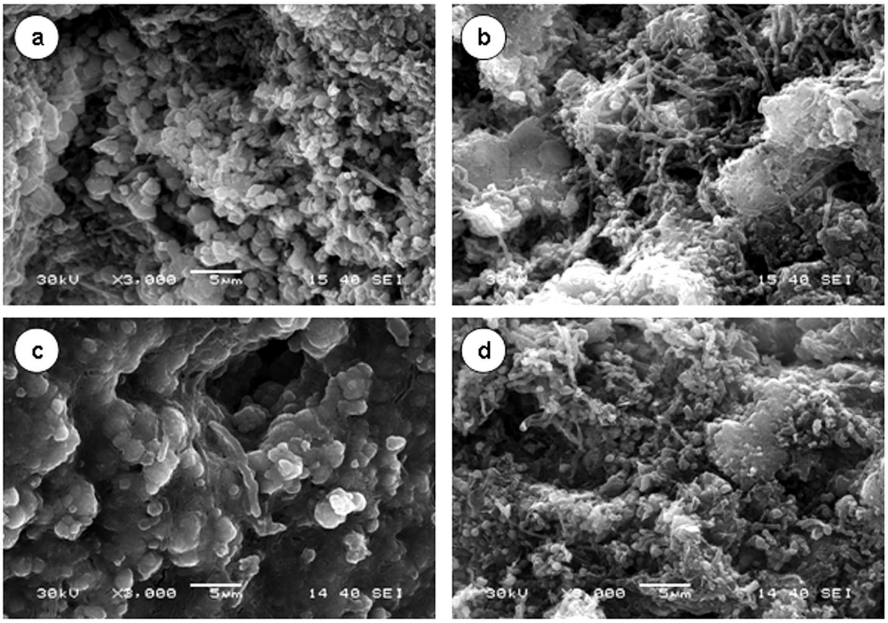

Fig. (3). Biofilm/dental plaque formation in the oral cavity. Biofilms were collected, fixed, and later examined under a scanning electron microscope. Biofilm formed on (**a**) acrylic resin, (**b**) a tooth surface, (**c**) dental enamel, and (**d**) gold amalgam.

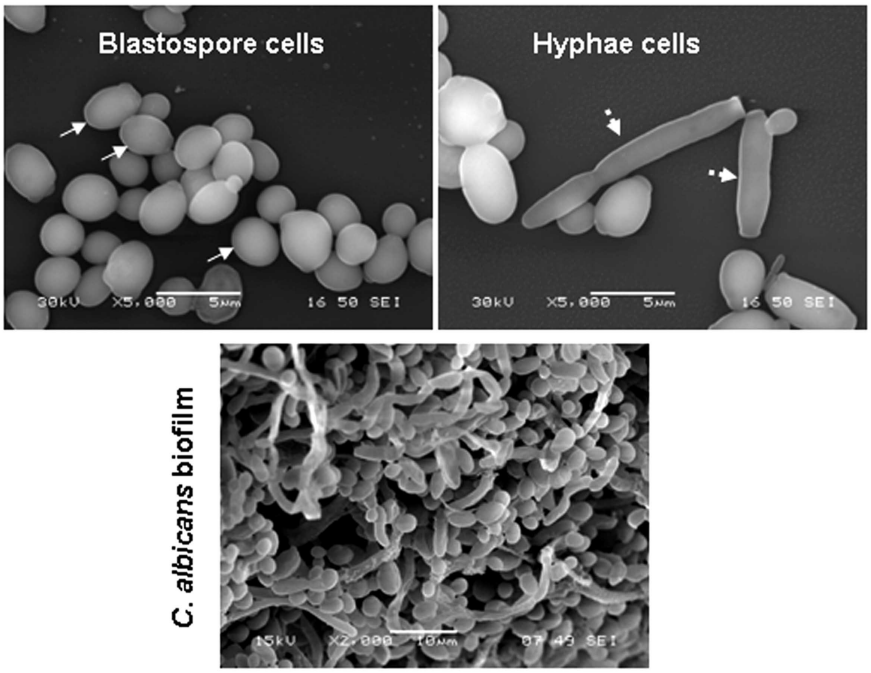

Fig. (4). Culture of *C. albicans* (Sc5314) as planktonic for 24 h and as biofilm for 4 days. Samples were prepared and later observed under a scanning electron microscope.

deeper into the tissue [30]. Gingival recession also provides favourable conditions for caries development. The gingival margin recession leads to exposure of the crown with the root surface, thereby facilitating biofilm formation and carious lesion development [31].

Various studies have established a clear link between caries and the quality of life of both children [32, 33] and adults. As teeth retention in the population increases, dental caries has become a burden for ageing adults worldwide [34-36]. In developing countries, this health issue is widespread in young as well as adult populations. For example, the dental caries prevalence in Tunisia was shown to vary between 48 and 58% in the young population (6-15 years old) [37] and young adult Tunisians are also affected. Indeed, Maatouk *et al.* (2001) showed that 70% of a student population aged between 18 and 22 years had caries [38]. Because biofilm is a major player in the disease, dental research must seek to understand the homeostatic mechanisms that maintain a normal and beneficial relationship between the resident oral micro flora and the host, thereby preventing biofilm formation. It is therefore crucial, to improve patient health, that appropriate procedures be designed to reduce biofilm-induced caries.

ETIOLOGY OF ORAL CANDIDIASIS

Widely distributed throughout the human population, *C. albicans* is a pleiomorphic fungus that can be either a commensal or an opportunistic pathogen [39]. *C. albicans* and some other non-albicans species are frequently found in the oral cavity of a vast number of populations ranging from children to adults/seniors. The oral carriage rate of *Candida* in healthy humans ranges from 40 % to 60% [40]. Areas of recovery of *Candida spp* in the oral cavity include the dentition, tongue, cheeks, and palatal mucosa, as well as from restorative materials and prostheses.

In healthy individuals, the presence of *Candida* rarely causes disease. However, under favourable conditions such as an immunosuppressed state, impaired salivary function, and the presence of foreign materials (dentures, prostheses), the presence of *Candida spp* may cause multiple candidiasis [41, 42] and is associated with root caries [43, 44].

C. albicans, the most commonly encountered yeast in infected patients, is a pleiomorphic fungus that has the ability to grow as yeast, hyphae, or pseudohyphae [45, 46]. This transition enhances its virulence and thus constitutes a key component in *C. albicans* pathogenesis [47, 48]. *C. albicans* transition from yeast to hyphal growth mode (Fig. **4**) contributes significantly to the penetration process into underlying tissue (systemic infection) and to biofilm formation [47, 48]. Biofilm ensures the survival of *Candida* in the oral cavity by conferring a resistance mechanism against therapeutic molecules [49]. The presence of *Candida* biofilms is associated with oral pathologies such as oropharyngeal candidiasis [50] and denture stomatitis [51].

Oropharyngeal candidiasis (OPC) is an opportunistic fungal infection involving various tissues, including the hard and soft palate, tongue, buccal mucosa, and floor of the mouth. *Candida*-infected tissue usually displays white curd-like lesions as well as reddened patches [50]. The leading cause of oropharyngeal candidiasis is *C. albicans* [52]. Due to the growing population of immunocompromised patients suffering from chronic diseases such as chronic inflammation, HIV infection, and cancer [53], the prevalence of OPC was increasing to a certain level. These health-threatening illnesses lead to changes in the oral ecosystem which favour *C. albicans* overgrowth, colonisation, and infection [52]. For example, up to 90% of HIV infected patients have had at least one episode of OPC [52]. Oropharyngeal candidiasis is associated with esophageal candidiasis [53, 54].

Candida is also associated with denture stomatitis. *Candida*-associated denture stomatitis was found in 60-65% of subjects wearing a removable prosthesis, with more diffused clinical manifestations [55]. In considering the subjects who manifested no clinical signs of inflammation, the percentage of *Candida*-associated denture stomatitis increased to 75% of the prosthesis-wearing population [55].

C. albicans has also been shown to be the principal *Candida* species responsible for inflammatory pathology through its ability to adhere to the hard and soft tissues of the oral cavity, proliferating and producing a complex and heterogeneous bacterial biofilm [56]. *Candida*-associated denture stomatitis is promoted by local and systemic factors related to the host [57]. Indeed, immunosuppressed states that accompany cancer treatments and organ or bone marrow transplants lead to changes in the oral ecosystem that also favour *C. albicans* overgrowth, colonisation, and infection [52]. If we are to successfully prevent infection and improve the health and well-being of patients, we must gain knowledge regarding biofilm formation and pathogenesis and the mechanisms of *Candida* resistance to antifungal agents.

ETIOLOGY OF PERIODONTAL DISEASES

The periodontium is a key structure surrounding and supporting the teeth. Any dysregulation of the periodontium results in periodontal disease. Inflammation, trauma, tumours, and genetic/metabolic changes are all forms of dysregulation that open the door to periodontal disease [58]. However, periodontal disease frequently refers to microbial inflammatory disorders involving the gums (gingivitis) and the periodontium (periodontitis).

Gingivitis is an inflammatory process encountered in the soft tissue surrounding the teeth. It occurs before periodontitis, affecting 50 to 90% of adults worldwide [59]. Patients who have gingivitis typically display inflammatory and bleeding gingival tissue. Gingivitis can be enhanced by different exogenous factors such as medications, hormones, nutritional conditions, and infection (biofilm) [60]. The most common type of gingivitis is a chronic form induced by biofilm that leads to periodontal disease. The prevalence, severity, and rate of periodontal disease may vary [61], with a reported prevalence ranging from 20 to 50% in the general population [62]. Similar to gingivitis, periodontal disease is frequently caused by oral microorganisms/biofilms. It has been reported that periodontal disease is associated with an increased density of gram-negative and anaerobic bacteria in the biofilm [63, 64]. It is interesting to note that bacterial count above the gums is higher than the bacterial level below the gums in a diseased periodontal pocket [65]. Bacterial level can be reduced contributing to biofilm reduction and maintaining periodontium health [66].

Periodontal disease is a serious health issue in the adult population worldwide. Studies have shown that bacterial species such as Porphyromonas gingivalis, Tannerella forsythensis, and the spirochaete Treponema denticola cohabit at subgingival locations and are associated with disease [67-69]. Bacterial damage to the periodontium thus occurs through direct contact and also via multiple bacterial mediators/ enzymes [70]. Furthermore, Actinobacillus actinomycetem comitans appears to be particularly involved in periodontal disease in young adults. It is also important to note that herpes viruses and C. albicans have been reported to play an active role in periodontitis [71-74]. Bacterial biofilms and bacterial toxins perturb gingival epithelial cells as the initial stage in a cascade of inflammatory and immune processes. This promotes the destruction of gingival tissues and ultimately alveolar bone resorption, leading to tooth loss [75].

It is also important to note that biofilm-related periodontal disease has been linked to multiple systemic illnesses, such as heart disease, premature births, and diabetes [76]. Thus future studies must investigate the relationship between biofilms and periodontal disease in order to ultimately develop new strategies to better control and prevent this disease, with direct positive repercussions on the health and well-being of millions of patients worldwide.

CONCLUSION

Microbial biofilms are critically involved in human pathogenesis due to their potential resistance to antimicrobial molecules. Indeed, one of the most important and intriguing properties of biofilms is their increased resistance to challenging environmental conditions. In particular, biofilms exhibit increased resistance to chemical disinfection, antimicrobial therapy, and human immune responses [77-79]. As an example, Fungi in a biofilm structure have been reported to be up to 1000-fold more resistant to antifungal agents than planktonic free-floating cells [80-82]. Technological advances have provided new tools to study microbial cells within biofilms, and as a result have produced some preventive/curative strategies [83]. However, research has yet to elucidate the key events involved in biofilm formation and drug resistance. These studies must examine the contribution of multi-species bacteria in the formation of biofilms as well as the role of beneficial microorganisms in this process. Further investigations are thus required to shed light on how microbial cells interact in mature biofilm. The more we are able to learn regarding this communication process, the sooner we will design and develop an innovative strategy to prevent and eradicate biofilm/dental plaque toward improving patient health and well-being.

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DECLARATION OF INTEREST

The authors declare no conflict of interest.

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