

Review Article

Biofilm Formation on Dental Materials in the Presence of Khat: Review

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Abstract

Microbiological composition in the oral cavity is affected by components and shape of restorative materials used. Consequently, such composition may affect oral health and restorative materials. Secondary caries form in teeth that are partly restored with restorative materials. This condition is a common dental disease caused by bacterial biofilms and with unknown causes. Caries are related to the type of restoration material used. In relation to biomaterials, several factors, such as surface roughness, surface energy, and chemical composition, affect Microbiota composition and biofilm formation. Ceramic and dental alloys have resulted in fewer caries formation, whereas composites cause more secondary caries than amalgam or glass ionomers. Khat chewing in the Arabian Peninsula is associated with a range of orodental problems. This paper provides an overview of scientific literature regarding the association among properties and performances of different restorative materials and oral biofilm formation in the presence of khat. PubMed literatures published until June 2016 were researched using the following keywords: ceramic, alloy, denture materials, composite resin, amalgam, biofilm, khat. Bibliographies of available previous reviews and their cross references were manually searched.

INTRODUCTION

The oral cavity is constantly contaminated by a complex diversity of microbial species that exhibit strong tendency to colonize dental surfaces, the tongue, and oral mucosa. The main components in biofilm formation comprise bacterial cells, a hard surface, and a fluid medium [1,2].

Formation of biofilms on intra and extra coronal teeth surfaces primarily causes periodontal diseases and caries [3]. A multitude of biomaterials used for restoration also cause such oral conditions [4].

Biofilm formation on restorative materials may degrade the material and roughen its surface [5], thereby causing filling of bacteria in the interface between tooth structure and restorative material and formation of secondary caries [6] and affecting pulp pathology [7].

Recovery of aesthetic and masticatory functions requires the use of proper dental restorative materials. However, these materials are prone to biofilm formation, thereby affecting oral health. In general, under clinical conditions, rough surfaces form more biofilm than smooth ones, but factors affecting bacterial adhesion to new restorative dental material remain unclear and

may result in increased synthesis of antimicrobial compounds [8,9].

Halbach [10], Luqman and Danowski [11] reported that long-term khat chewing causes stomatitis followed by secondary infection. This finding may be caused by chemical irritation of mucosal surfaces and mechanical strain on cheeks and other oral tissues. Low prevalence of dental caries and high rate of periodontal pocket depth and diseases have been reported among khat chewers [11].

Recently, the effect of khat on oral bacteria has been assessed in a series of studies. *In vitro* experiments showed that crude khat extracts interfere with biofilm formation by *Streptococcus mutans*, suggesting their anticariogenic properties [12]. In another study, extracts exhibited selective antimicrobial properties against major periodontal pathogens [13] and were found to foster growth of some health-compatible species [14]. The present paper aims to highlight the association between physical and mechanical properties of restorative and prosthetic dental materials and oral biofilm formation in the presence of khat.

THE BIOFILM FORMATION

Dental biofilms are matrix-enclosed bacterial population

adherent to each other and/or to surfaces, including polished tooth surfaces, living tissues, prosthetics devices, and dental materials. These films provide colonizing species with advantages, such as protection from competing microorganisms, environmental factors, host defense, and toxic substances. In the oral cavity, dental biofilm comprises diverse microorganisms; more than 500 different cultivable bacterial species are indigenous to the human oral cavity [15,16]. Currently, more than 700 oral bacterial taxa have been identified [17]. Among these organisms, approximately 100 or fewer species normally inhabit the oral cavity of an individual [18]. Although whole saliva features no distinctive microbiota of its own [19], it harbors as much as 108 bacteria per 1 mL [20] and serves as reservoir of microorganisms regularly derived from dental plaque biofilms adhering to gingival crevices, periodontal pockets, the dorsum of the tongue, and other oral mucosal surfaces [21]. Using only sequence analysis of previously characterized 16S rDNA and a number of previously uncultured and uncharacterized ones, bacterial species have recently been identified in saliva of healthy individuals and patients with periodontitis [22]. *Mutans streptococci* and *lactobacilli* have been associated with etiology of dental caries. Among *mutans streptococci*, *Streptococcus mutans* and *S. sobrinus* are considered particularly significant in human caries [23].

BIOFILM FORMATION AND KHAT

Khat is the leaves of the shrub (*Catha edulis Forsks*) which are widely spread, chewed, consumed, and practiced by a majority of the youth in Jazan southwest (Saudi Arabia) [24,25]. Khat chewed like tobacco or used to make tea daily or during social and cultural gatherings and held in the lower buccal pouch unilaterally in a bolus for more than 5 hours or more [26,27]. Khat was reported to cause dental attrition, staining of teeth, TMJ disorders (pain and clicking), and cervical caries particularly among crystallized sugar consumers, and increased periodontal problems and attachment loss [28].

An *in vitro* effect of crude khat extracts on oral micro-organisms and the effect of bacteria identified from sub and supra-gingival plaques. Al-Hebshi et al. [14], demonstrated a possible antimicrobial effect of khat on oral micro-organisms and showed a selective antimicrobial effect of crude khat extracts on oral micro-organisms. They demonstrated that while bacteria associated with periodontal disease were sensitive to the extracts, bacteria associated with periodontal health were less sensitive, and cariogenic bacteria were not susceptible. In another study, Al-Hebshi et al. [12], showed that crude khat extracts interfered with the ability of *Streptococcus mutans* to form adherent biofilms, implying that khat may have anticariogenic effects.

Nyanchoka et al. [29], founded a significantly higher caries rate in khat chewers than in non-chewers, as measured by the decayed, missing and filled teeth (DMFT) index. They found the mean DMFT score were 8.778 and 6.529 for chewers and who never chewed khat respectively. The authors suggested that the higher caries index score in chewers could be a result of cariogenic substances such as soft drinks that are often consumed with khat [29,30]. While Hattab and Al-Abdulla [26] noticed that, Khat leaves contain a negligible amount of fluoride and thus is unlikely to exert anti-caries effect as claimed previously.

Regarding the effect of khat chewing on oral micro-organisms, the available evidence consistently indicates that chewing khat did not favor the proliferation of pathogenic oral micro-organisms. It was shown to have selective antimicrobial effects and to favor the presence of micro-organisms compatible with oral health [31,32]. A studies by [31-33], concluded that Khat chewers shown periodontal health adverse outcomes such as, gingival recession or bleeding and periodontal pocketing comparing to non-chewers, with effect sizes ranging from medium to large. It has also been shown that chewing is associated with other indicators of periodontal health and tooth loss [34].

BIOFILM FORMATION AND PROSTHETIC MATERIALS

Prosthetic materials may affect accumulation of biofilm in different ways. Rough or open margins consistently form between tooth and prosthesis, and this condition may complicate mechanical removal of biofilms and alter chemical balance in biofilm in this region.

Ceramics

The use of dental porcelain is advocated in different types of restorations like veneers, inlays, single crowns and fixed partial dentures [35]. Studies both *in-vitro* [36,37] and *in-vivo* [36,38-42] have investigated the adhesion of bacterial and bacterial biofilms on ceramics in comparison to other dental materials. Relatively and in comparison to other dental materials used in oral cavity, ceramics have been found to promote lower bacterial adhesion and biofilm formation although very less *in vivo* studies have been conducted to study the differences between different types of ceramics [43,44]. Variation between different ceramics has been studied *in vitro* for determination of bacterial adhesion rather than the accumulation of complex biofilms [44]. Ceramic surfaces have been shown to collect less plaque with reduced viability in absence of oral hygiene although different results have been demonstrated when compared with unglazed porcelain surface [45,46].

Acrylic resins

Since 1928, denture base resins are a group of dental materials that have stood the test of time without undergoing much change in its basic constituents. Biofilm associated with denture base resins is unique in the sense that more than bacteria, it is certain yeasts especially candida species that have been associated with denture base resins [47-49]. Many different strains of candida [50] along with certain bacteria [51] have been shown to work synergistically for their attachment to denture base resin or to each other [49, 52,53].

Biofilm including yeasts has been found to be difficult to remove because of strong adhesion ability, [54] the adhesive ability is directly associated with microporous surface of denture resins [55-57].

Modification of resins to discourage biofilm formation in the form of polyethylene [54], titanium dioxides coating [58] and denture cleansers [59] have shown to discourage the biofilm formation.

Metal alloys

Alloys used for prostheses should be inert and highly polished to prevent the accumulation and attraction of oral micro-organisms to prevent biofilm formation. Different alloys used in dentistry mainly gold, nickel chrome and recently titanium alloys.

Prostheses alloys margin with many small defects will retain more plaque and bacteria than a smooth margin. Most alloys should be polished to give very little retention for biofilms, although some alloys have a higher affinity to bacteria than others [60]. It seems that some bacteria are attracted due to electrical charges in some alloys [8]. Biofilms on gold restorations, however, generally have low viability [60]. Some microbes are affected by elutes from the metals.

Auschill et al. [60], demonstrated that oral biofilms have very low viability (less than 2%) on gold but this cannot be due to the release of toxic compounds, because gold is completely inert. They explained that possibly, full coverage by a relatively thick oral biofilm hampers the supply of nutrients to the biofilm, leading to low viability [61].

Biofilm formations and restorative materials

Dental restorations affect biofilm composition in different ways. Steps, open margins, or groves consistently form between tooth and restorative materials. These spaces will complicate mechanical removal of biofilms and alter chemical balance in the biofilm in this region. Restorative materials differ from enamel with regard to surface roughness, surface energy, and chemical composition [62,63]. Most populations receive at least one dental restoration, and roles of biofilm-related infections to restoration as opposed to primary oral infections are not easily distinguished.

Amalgam

This material cannot bond to the tooth structures, so it depends mainly on macro-mechanical undercuts for their retention. This resulted in interfacial spaces which lead to secondary caries [64]. Since amalgam is a conducting material, like gold so, electron transfer plays a role in bacterial adhesion [65]. This is attributed to attraction between the negatively charged bacteria and their conducting material positive image charges [66]. Auschill et al. [60], ring biofilm on amalgam and gold and found that five-day-old oral biofilms on their surfaces were thick and fully covering the sub-stratum surfaces [60]. Leonhardt et al. [67], placed different restorative materials in teeth for day and 3 days, he showed that amalgam attracted about 50% of viable bacteria than titanium oxide [67,68]. They explained the low viability of biofilms on amalgam surfaces is may be due to the release of toxic compounds from the alloy. However, it is possible that bacteria develop resistance against mercury because of instant bacteriostatic effects of it [60]. Experimentally more bacteria resistant to mercury were found in microcosm oral biofilms grown on amalgam than on enamel. The percentage and levels of this mercury resist bacteria remained elevated for a period of 2 days, but after that it returned to baseline levels [68].

Composite resins

Surface deterioration of resin composites has been demonstrated by an increased roughness, effects on filler

particle exposure, and sometimes by a reduced micro-hardness of the materials upon exposure to biofilms *in vitro* [5]. Clearly, the clinical presence of biofilm is just one of the factors that may stimulate surface degradation, other factors being acidic fluid intake, temperature fluctuations, or simply the presence of an aqueous environment.

Some methods to inhibit biofilm growth on dental material are such as blended the zinc oxide nanoparticles into resin composites and or adding of chlorhexidine gluconate into some dental materials in order to enhance the antibacterial activity and display antimicrobial activity and reduce growth of bacterial biofilms [69-71]. In addition to that development of a nano-composite containing amorphous calcium phosphate or calcium fluoride nanoparticles and chlorhexidine gluconate particles might be reduced biofilm formation Cheng et al. [72].

The removal of filler particles based on the roughness dimensions created. Resin composites with larger 0.01 to 3.5 μm filler particles became significantly less rough (around 15 nm) after biofilm growth [5].

Recently Khalichi et al. [73], found that triethylene glycol, as the ether portion of triethylene glycol dimethacrylate, modulates the expression levels of glucosyltransferase B involved in biofilm formation and *yfiV* as a putative transcription regulator gene in *S. mutans*.

Glass-ionomer fillings

Biofilm formation on glass-ionomer cements leads to a negative spiral of events [5], in which the colonizing organisms cause severe deterioration of the surface, which, in turn, promotes biofilm formation and therewith more extensive deterioration of the surface. The clinical manifestation of this downward spiral is the development of caries around or below a restoration [74].

The use of glass-ionomer potentially reduces micro leakage by adhering to tooth structure and enhances fluoride release with a potential impact on oral biofilm formation. Fluoride release occurs through an initially high burst release that may be between 1.6 and 1.8 $\mu\text{g}/\text{mm}^2$, after which a prolonged, long-term tail-release follows [75].

Fluoride can act as a buffer to neutralize acids produced by bacteria and reduced the growth of caries related oral bacteria [76,77]. Glass-ionomer cement indeed collects a thin biofilm with a low viability (2% to 3%), possibly as a result of fluoride release [60]. However, an *in vitro* study also showed that glass-ionomer containing fluoride did not reduce the amount of bacterial growth and biofilm formation on the surfaces bathed in saliva [78]. This suggests that either fluoride is not a dominant factor in controlling biofilm formation, or the too low concentration to be effective, depending on the ratio between filling area and fluid volume in which the experiments were carried out. In the oral cavity, the large volume of saliva present, which is subject to wash out, makes the build-up of an effective fluoride concentration difficult [75].

Association of biofilm and khat on prosthetic and restorative materials

No study explored the relation of prosthetic materials

and biofilm among khat chewers or the relation of khat and restorative materials. Little information is available on the pattern of dental biofilm distribution on different Prosthodontics and restorative materials. However, no literature or clinical and laboratory studies demonstrate the relationship between biofilm formation and khat chewers.

DISCUSSION AND CONCLUSION

In vitro and *in vivo* studies reveal that rough surfaces will promote plaque maturation and formation on restorative materials. Thick biofilms form on metal alloys and amalgam, but thin ones are more common in ceramic and glass ionomer restorations. Khat chewing has been shown to modify compositions of supra- and subgingival microbes, leading to periodontal recession, pocketing, and attachment loss of teeth. Case control and high-powered cohort studies bear significance in investigating the association among biofilm formations, restorative materials, khat chewing, and dental health. Finally, the present review should be considered for further clinical studies.

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