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Systematic Review

Biofilm in dental biomaterials: A review

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ABSTRACT

All dental treatment using biomaterials in the body can affect the host in both positive and negative ways. Oral biofilms attach onto both teeth surfaces and dental material surfaces in oral cavities. This may impair oral health as well as general health of the patient.The composition of the microbiota and the formation of biofilm in relation to biomaterials such as, surface roughness that provide favourable interface for bacterial colonization. Moreover, the factors like surface free energy, hydrophobicity and chemical composition also have a significant influence on the formation of oral biofilms. The aim of this review article is to give an overview of the scientific literature regarding the association between the properties of dental biomaterials and oral biofilm formation, with emphasis on current research and future perspectives.

Keywords: Amalgam; biofilm; biomaterials; composites; glass ionomer cement

1 INTRODUCTION

Oral biofilm mostly contain harmless microorganisms, with some have the ability to cause damage to the teeth as well as infections in the soft tissues. Caries are the primary reason for less than half of the dental restorations produced annually.[(1)] Majority of restorations placed in day to day dental practices are by the replacements of old restorations, due to biofilm related secondary infections. By inserting foreign bodies such as dental restorations, increases the number of pathogenic microorganisms and thereby increase the risk of developing diseases like secondary caries along a restoration, complete denture associated fungal infection or periodontitis on teeth supporting a partial denture.

The oral environment,has high humidity, moderate temperature, and abundance of nutrients promote the formation of differentiated microorganisms and microbial biofilms.[(2–4)] Biofilm formation in the oral cavity is a gradated process consisting of four stages (Figure 1)[(5)]:

1. Acquired pellicle formation
2. Primary colonization
3. Coaggregation
4. Mature biofilm establishment.

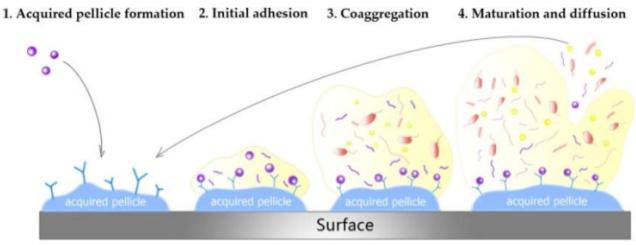

Fig. 1: The process of biofilm formation in the oral cavity is divided into four stages: 1.acquired pellicle formation; 2. initial adhesion; 3. coaggregation; 4.maturation and diffusion.

To generate a biofilm, all surfaces exposed to the oral environment are steadily covered by a pellicle derived from the adsorption of organic and inorganic molecules in saliva. The receptors of salivary pellicle offer binding sites for floating initial bacteria cells to attach to these surfaces and form microcolonies. As time goes by, the bacteria cells

aggregate, proliferate, and grow into a mushroom-shaped mature biofilm, firmly attaching to these surfaces.[6,7] Therefore, bacterial cells within the biofilm do not exist as independent entities but, rather, as a coordinated, metabolically integrated microbial community.[5]

2 ORAL BIOFILM

In the human oral cavity there are hundreds of different species of microorganisms, including bacteria, virus and fungi.[8] More than 700 unique bacterial species have been detected.[9] There can be more than 10^{11} microorganisms per mg of dental plaque.[9,10] These live in complex societies, usually organized in thin layers covering the oral surfaces—biofilm—for example as dental plaque on tooth surfaces.[11,12] Immediately after cleaning, the proteins from saliva will cover the tooth surfaces in a pellicle. Bacteria attach to this pellicle by microfilaments in their cell walls. When the bacteria increase in number, they will be able to communicate by secreting signal molecules and create a community.[11,13] The bacteria secrete proteins, polysaccharides, nucleic acids and other substances to the extracellular matrix, additionally containing proteins and nutrients from saliva. This matrix is the "glue" of the biofilm. The microorganisms inside a matrix behave differently from bacteria floating freely in the saliva (planktonic growth). The biofilm community behaves as a unit in response to environmental changes rather than as single bacterial responses. The matrix protects the organisms inside from chemical treatment that could have been lethal for planktonic bacteria, for example antibacterial mouth rinses.[14] Complete removal of oral plaque is difficult due to limited access between teeth and in deep crevices on tooth surfaces. Dental restorations with pores, gaps and margins further complicate this. In healthy individuals the biofilm on teeth will function as a protective shield from foreign microorganisms and chemicals in the food, such as acids that can potentially dissolve dental enamel.[15] If, however, the biofilm is left untreated, an ecological shift might occur, favoring microorganisms that may have detrimental effects on teeth, surrounding tissues and the patient's oral and general health.

The thickness and structure of a biofilm will be affected by many factors, such as pH, nutrients, oxygen, time since last cleaning and the kind of surface to which it is attached.[12,16–21] The biofilm in the oral cavity will therefore differ in the different locations, such as on the cheek or in between teeth. A biofilm that is allowed to grow over days will have a different composition than a biofilm that is mechanically removed and renewed daily.[12] The bacteria adjust to the matrix and surrounding organisms by gene regulations. Biofilm formation is a big problem for all medical biomaterials in humid environments, such as artificial heart valves, artificial vocal cords and incubation tubes.[3,22] They often have the added complications that access for cleaning procedures is impossible. However, they are usually not in environments with such an abundance of microorganisms as in the oral cavity.

2.1 2.Oral microorganisms and oral infections

Growth of pathogenic microorganisms can lead to disease if the host's immune response is not able to neutralize or destroy them.[13] The most common oral infections are pulpitis from untreated caries, gingivitis, and periodontitis.[12] The microorganisms in oral biofilm are mainly bacteria. However, adults usually have both fungal and viral species present in the oral microflora.[8,9] Candida albicans will normally be present only in small amounts in healthy adults because the healthy biofilm favor other microorganisms. The microflora develops gradually as the environment in the mouth changes. Microorganisms with and without known ability to provoke diseases will be present in the flora. As long as the majority of microorganisms are non-pathogenic, the host stays healthy, but if the conditions favor growth of pathogens, disease might develop.[9]

Teeth are unique organs since they penetrate the oral mucosa and have one part secured in bone and one part exposed to the oral cavity.[23] The exposed part is continually colonized by the oral microorganisms. The periodontal pocket surrounding the tooth is designed as a protective barrier against the invasion of microorganisms to the mucosa and the alveolar bone. The cells in the pocket release exudate with antibacterial effect that hinders microorganisms from invading the pockets. Increased thickness of the biofilm will reduce the pH-, oxygen- and nutrient-levels in the bottom layers of the plaque.[12,13,16,21,24] Food debris in the biofilm will ensure access to nutrients for the bacteria. This will be favorable for the species associated with dental caries (Streptococcus mutans, Actinomyces spp., Veillonella spp., Lactobacillus spp.) and gingival and periodontal inflammation (Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia etc.).

Caries is in itself not an infection, but demineralization of dentin and enamel. The demineralization occurs as a consequence of acid production from bacteria in the biofilm when they process sugars in plaque.[25] The biofilm becomes acidic (pH < 5) and the hydroxyapatite in the enamel dissolves. When the lesion is very deep, the pulp of the tooth might be infected with oral bacteria and inflammation occurs, known as pulpitis.[12] Gingivitis occurs when the biofilm is too thick to be effectively wash away by the gingival exudate. The anaerobic bacteria are favored and intrude into the periodontal pocket. Proliferation of pathogenic bacteria in the pocket induces inflammatory response in the gingiva with swelling and increased bleeding which complicates the cleaning procedures. This response can in turn dissolve the alveolar bone supporting the teeth—periodontitis—which eventually will result in tooth loss.[10–12,16]

Oral infections can affect the hosts' general health in many ways.[10,25] Firstly, it is unfavorable to have ongoing infections in general. Oral infections such as gingivitis can involve quite large areas, and is a constant burden for the hosts' immune system. This is, of course, most harmful for patients with other ongoing inflammations or diseases. Secondly, oral microbes may spread to other organs within the host either via the respiratory system or through the blood stream.[10–12,24–26] This is, again, most harmful for patients with other complications, such as artificial heart valves, or transplanted organs. Elderly people with pneumonia will often have oral microbes in their lungs, which may have been aspired from the oropharynx and caused the inflammation in the lungs.[10,24,26] The direct causality between oral infections and systemic disease has been difficult to prove. It is difficult to detect whether the oral microorganisms were present prior to the disease and were the direct cause of the infection or not. It is also possible that the oral microbes entered the loci after the primary inflammation occurred due to reduced effect in the immune response system. Candida albicans can spread directly from the oral cavity to the throat and stomach.[27,28] General candida infections are extremely difficult to cure due to the high risk of re-colonization. Thirdly, individuals with poor general health will normally also have poor oral health, due to malfunctioning immune system and altered chemical and physical conditions in the body, such as reduced saliva flow, iron deficiency, malnutrition, medical treatment, etc..[29,30] Sick and elderly have reduced ability to clean their teeth properly and are therefore more exposed to development of unfavorable biofilm and subsequent general infection.[31]

2.2 *Biomaterials' effect on biofilm*

Dental restorations affect the composition of the biofilm in many ways. There will always be steps, gaps or groves between tooth and restoration. These will complicate mechanical Biofilm removal and alter the chemical balance in the biofilm in the region.[32,33] Restorations differ from enamel with regard to surface roughness, surface energy and chemical composition.[32–36]

Many different studies have been performed evaluating the effect on biofilm formation from surface qualities such as surface energy, roughness, topography and chemical composition of the restorative materials.[37–39] Both surface quality and chemical composition will affect the topography and surface energy and the different studies do not always disclose or differentiate properly between the different factors. Multiple factors are working simultaneously that all may affect the outcome. Comparison between different studies and among materials is not straight forward. Furthermore, the findings from in vitro and in vivo studies are not always in coherence with each other, which indicates that the oral biofilms in vivo are complicated and difficult to mimic in a laboratory setting. Most studies evaluate only a selection of pathogenic bacteria or only one surface variable.[9,40] As discussed above, it is not likely that one surface quality or single microorganisms are the cause of disease alone but rather a shift in the composition in the biofilm.

3 PHYSICAL CHARACTERISTICS OF DENTAL MATERIALS

3.1 *Surface roughness*

Nowadays, some clinical procedures, polishing and finishing, are usually applied for smoother surfaces. Among these polishing and finishing techniques, the lowest surface roughness (SR) values could be achieved by Mylar, and followed by Al_2O_3 discs, one-step rubber points, diamond bur, and multi-blade carbide bur.[41] Many researches have demonstrated that unpolished materials surfaces could accumulate more dental biofilm than polished ones, including resin-based composites, ceramics, implant abutments, and denture bases. Kim[42] investigated the surface ultrastructure, roughness of four ceramic materials (Vita Enamic, Lava Ultimate, Vitablocs Mark II, and Wieland Reflex), and assessed their promotion of biofilm development following adjustments and simulated intraoral polishing methods. A maximum surface roughness of Ra = 0.2 μm has been suggested as a threshold value for bacterial retention. Below this value, no further reductions were observed, while over this value, biofilm accumulation increased with increasing roughness.[37] Surface roughness can, however, be measured in many ways. Ra gives an arithmetic mean of the surface roughness.

The deeper and larger depressions may increase the contact area and provide more favorable interfaces for bacterial colonization and biofilm formation, protecting bacteria against shear forces (rinsing and brushing) during their initial reversible binding, leading to irreversible and stronger attachment.[43,44] Hence, it is difficult to eliminate microcolonies on the rough surfaces, resulting in the formation of mature biofilm.[45]

3.2 *Surface energy*

Surfaces with a low surface energy usually display lower adherence to biofilms than similar surfaces with higher surface energy.[38] No effect of changes in surface energy was found in a study on surface nano roughness, texture and chemistry.[46] Most dental materials, with the exception of ceramics, have a higher surface energy than enamel and have thus a greater risk of biofilm accumulation. Alteration in surface roughness will in most cases also alter the surface energy. It is therefore difficult to distinguish between the two factors. It seems that surface roughness plays a more important role than surface energy.[39]

4 CHEMICAL COMPOSITION OF DENTAL MATERIALS

The chemical composition of the dental material will further affect the bacterial adhesion since both proteins and microorganisms can chemically attach or attract to components in the material, by van der Waal forces, acid-base reactions or electrostatic interactions.[47] In most patients, there will be several different materials present in the mouth simultaneously with can interfere with the biofilm formation and the microbiota in general. The chemical interaction between material and microorganisms can lead to alterations in the surface properties over time.

4.1 Polymers –COMPOSITES

Recently, it was discovered that RBCs with a UDMA/aliphatic dimethacrylate matrix blend showed significantly higher biofilm formation on the surfaces than specimens with a BisGMA/TEGDMA matrix blend and analogous filler fraction, except for nanosized filler particles.[48] Another matrix, the silorane-based composite, was demonstrated to be less prone to *S. mutans* biofilm development compared with a generally used methacrylate-based composite, due to the increased hydrophobicity by silorane.[49] There are different sized inorganic fillers of the resin composites, including macrofill, microfill, nanofill, and hybrids. The RBC's strength and polishing ability mostly depend on the size and proportion of inorganic fillers.[50] Pereira et al. demonstrated the least biofilm formation on a nanofilled RBC (Filtek Z350^{TM}) compared with nanohybrid, microhybrid, and bulk-filled RBCs.

Up to now, there is still a high secondary caries rate, probably because of relatively few commercially antibacterial resins materials applied in clinic. However, more and more experimental antibacterial components and materials have been produced in the lab,[51–54] among which, 12-methacryloyloxydodecylpyridinium bromide (MDPB), fluoride, and nanoparticles, have been translated into clinical materials. Both experimental antibacterial materials and new commercial antibacterial materials will soon pioneer a new materials field.[55–57]

4.2 Glass ionomer cements

Glass ionomer cements (GICs), applied as direct restorative materials and cements, feature some desirable characters, such as a chemical adhesion to enamel and dentin, and the ability to release fluoride over time.[58] It is well known that conventional GICs have biological effects and caries-inhibiting properties because of the release of surface fluoride ions.[59]

Recently, many studies have reported that the fluoride of GICs can affect the acid production, acid tolerance, and extracellular polymetric substance (EPS) formation of dental plaques, especially cariogenic biofilms, such as *S. mutans* biofilms. The fluoride can reduce the proportion of *S. mutans* but increase S.oralis(Streptococcus oralis) in the dual-species biofilm, subsequently inhibiting the formation of cariogenic bacteria-dominant biofilms.[60]

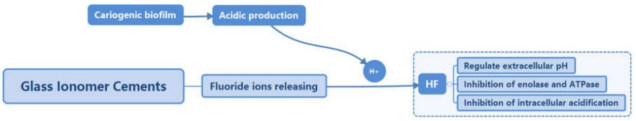

Fig. 2: The relationship between fluoride of glass ionomer cements and bacterial metabolism.

4.3 Amalgams

Over its long clinical history, dental amalgams have evolved and served the profession successfully and at low cost. Amalgam restorations are being phased out because of the environmental pollution and inferior esthetic appearance.[61] However, they cannot be replaced by other restoratives because of their perfect mechanical properties, longevity, and low cost.[62] After clinical placement, amalgam restorations undergo a series of corrosion to release a variety of metallic ions in oral cavities In the 1980s, the amalgam was proved to have bacteriostatic and bactericidal properties due to the metallic ions being released from the surface of the materials, such as Ag, Cu, Sn, and Hg.[63] The low biomass of oral biofilms on amalgam surfaces is probably a result of the release of toxic ions from amalgam, which mainly consists of Hg and Ag.[64] Specifically, amalgam showed lasting inhibition of both *S. mutans* and *Actinomyces viscosus* (A. viscosus) which played crucial roles in biofilm formation.[65] Morrier et al. investigated that the order of antimicrobial potential of elements in amalgams would be Hg > Cu > Zn, by testing a suspension of *S. mutans* and *A. viscosus*.[66] This can be related to the fact that biofilms accumulated more on composites than amalgams in the clinic.

4.4 Dental alloys of indirect restoration

After 1975, the alloys for full-cast restorations, porcelain-fused-to-metal restorations, and removable partial denture frameworks, can be divided into three kinds, high-noble alloys (Au–Pt, Au–Pd, Au–Cu–Ag–Pd), noble alloys (Au–Cu–Ag–Pd, Pd–Cu, Pd–Ag), and base-metal alloys (Ni–Cr, Co–Cr, Ti).[67] Oral microbial metabolites, such as acids, sulfide, and ammonia, can induce the microbial corrosion of metallic materials.[68] Dental alloys corrode and release metal irons in the oral environment which may compromise material biocompatibility and mechanical properties, and lead to the esthetic loss of dental restorations, and influence health.[69]

Among the noble alloys, a high gold content alloy (88% by weight), Captek^{TM}, showed a 71% reduction in total bacterial

numbers when compared to natural tooth surfaces.[70] This could be attributed to the low porosity of high nobility gold inherent in the manufacturing process and the unique electrochemical corrosion resistance.[71] Besides, metallic copper and copper-containing alloys possess a strong and rapid bactericidal effect, named "contact killing". This was induced by successive membrane damage, oxidative damage, cell death, and DNA degradation.[3,72] The surface-released free copper ions are toxic to bacteria because of their soft ionic character and their thiophilicity.[72,73] As for the base-metal alloys, a higher amount of viable microbial cells and biofilm density on prosthetic structures based on cobalt–chromium (Co–Cr) alloys was demonstrated, when compared to those based on titanium.[4,74] Zhang et al. discovered that corroded alloy surfaces could upregulate gene expression of the glucosyltransferase BCD, glucan-binding proteins B, fructosyltransferase, and lactate dehydrogenase in *S. mutans*, which play critical roles in bacteria adherence and biofilm accumulation.[68]

4.5 Ceramic

In recent years, adhesively cemented ceramic restorations, such as inlays/onlays, veneers, and crowns, have been used as the main approach for minimally invasive esthetic restorations in anterior and posterior teeth.[75] However, its clinical failure is related to a lot of factors, such as marginal misfit, surface irregularities, and cement excess, which may favor the accumulation of microorganisms, compromising clinical restoration longevity.[76]

Both surface roughness and surface free energy have been found to influence initial microbial adherence decisively[77], due to compositional and microstructural differences, and bacterial colonization was thought to differ from one ceramic material to another. Zirconia exhibited low biofilm accumulation.

4.6 Dental implant

Over the last decades, the use of dental implants has become a common way of restoring dentition defect.[78] The implant survival rate reaches to 92.8–97.1% over a follow-up period of 10 years, but dental implants easily become infectious, due to oral pathogenic bacteria.[79–81] Two main etiologies of peri-implantitis are oral biofilms and occlusal overload,[82] among which, oral biofilms developed on dental implants play a significant role in peri-implantitis' pathogenesis. The peri-implantitis can cause implant loss in the absence of prevention and therapy.[83,84] The implant may be attached by saliva, blood, and oral bacterial cells during and after the implant surgery, and bacterial cells attached to the abutment harm the surrounding gingiva. All the above-mentioned points would affect the healing and restoration following surgery.[85]

It has been found out that, except for surface roughness and surface free energy,[86] the type of the biomaterial itself can also influence biofilm formation and subsequent plaque accumulation on implant surfaces. Zhao's[87] study showed that neither roughness nor hydrophobicity had a decisive influence on the biofilm formation that occurred on three different implant materials, comprising titanium (Ti, cold-worked, grade 4), titanium–zirconium alloy (TiZr, 15% (wt) Zr) and zirconium oxide (ZrO_2, Y-TZP). Same as Zhao's result, in the 3-species biofilm (Streptococcus sanguinis, Fusobacterium nucleatum, and Porphyromonas gingivalis), the analysis showed that there were no significant differences between titanium and zirconia in terms of total biofilm mass and metabolism. However, zirconia revealed significantly reduced plaque thickness. Regarding human plaque biofilms, microbiological techniques showed statistically significant reduction in biofilm formation for zirconia compared to titanium. The result suggested that not only surface roughness or surface hydrophilicity might be important factors for biofilm formation, but also material composition—metals compared to ceramics—suggesting a reduced disposition for peri-implant plaque and subsequent potential peri-implant infections on zirconia compared to titanium implant surfaces.[44,88]

5 CONCLUSION

As discussed in this review, bacterial adhesion and biofilm formation can be strongly influenced by surface characteristics of dental materials, which include chemical compositions, surface roughness, surface free energy, surface topography, ions release, and others. In conclusion, every possible particular chemical composition (organic matrix, inorganic filler, fluoride, and various metallic ions) can enhance or inhibit biofilm formation. Irregular topography and rough surfaces provide favorable interfaces for bacterial colonization, protecting bacteria against shear forces during their initial reversible binding and biofilm formation. Besides, the surface free energy, hydrophobicity, surfaces coating techniques also have a significant influence on oral biofilm.

Due to the many problems associated with biofilm formation on dental biomaterials, the challenge is to improve the materials in use. Reduced polymerization shrinkage of dental resin composites is believed to reduce the leakage of microorganisms into the gap and thereby reduce the rate of secondary caries, although the clinical relevance is difficult to detect.[89] Fluoride release from glass ionomer cements are meant to reduce the demineralization of the dentine and enamel on the tooth adjacent to the cement.[90] Fluoride release from other polymers have not been equally successful. Lately, there have been many attempts at altering the chemical composition in the materials with the intention of reducing biofilm formation.[2,91,92] Different strategies are used to achieve this, mainly reducing the initial attachment

of bacteria by altering the surface properties or reducing the viability of the attached bacteria by chemical components.[2] Some dental materials include monomers with antibacterial eluates .[93] Antibacterial chemicals, such as chlorhexidine or silver nanoparticles, can be embedded in nanoparticles in resin, coatings, acrylics, sealers or cements.[94,95] The nanoparticles dissolve slowly from the material and destroy the bacteria in the biofilm attached to the surface of the material. More than 50 abstracts each year concerning antibacterial effects of dental restorative materials have been presented in the International Association of Dental Research meetings worldwide in recent years, illustrating the current interest for this clinical problem. There are several potential benefits from including slow releasing antibacterial particles in biomaterials. The number of bacteria in the biofilm can be reduced, the potential harm from biofilm formation can be reduced, and the oral environment be made healthier. The antibacterial effect can be used in specific localizations, such as in the layer between filling and tooth, where they are most effective and thus reduce the general effect on the patient.[96,97] Antibacterial liners can ensure that remaining bacteria in the caries lesion are killed and thereby arrest the development of the lesion without excessive removal of tooth substances.[96,97] Antibacterial sealers for endodontic treatment can probably reduce the number of visits and increase success rates of endodontic treatment.[98] Antibacterial surface modifications or coatings are investigated for a wide range of applications also outside medicine and dentistry.[2]

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