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REVIEW

Bacterial colonization of composite resins used with direct technique. A brief review.

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ABSTRACT

In restorative dentistry, the use of composite resins with direct technique for the replacement of missing tooth structure is very common. One drawback is that surface roughness allows the adherence of microorganisms and the formation of dental plaque, being the polishing technique a key stage in the restoration process. The aim of this paper is to review the process of bacterial colonization of composite resins used with direct technique. According to in vitro studies, bacterial adhesion on microhybrid composite resins is 3.91 ± 0.52 UFC and on nanohybrid is 3.34 ± 0.74 UFC. Resins with particle size of 2.5 micrometers contained a greater volume of biofilms and enabled adhesion of S. mutans; in turn, resins with particle size of 0.1 to 0.4 micrometers showed lower bacterial adherence. As summary, the degree of bacterial colonization depends on hygiene, polishing technique and composition of restorative material: the bigger the particle size, the greater the adhesion of bacterial plaque.

Keywords: *microorganisms, resin composite, roughness.*

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INTRODUCTION

In the human oral cavity there are more than 700 unique bacterial species, such as Streptococcus *(S. mutans, S. sobrinus, S. sanguinis)*, Lactobacillus *(L. casei, L. Fermentum, L.oris, L. plantarum)* and Actinomyces *(A.israelis, A. naeslundii)*, among others. The most frequent on the colonization of resins are S. sobrinus, S. mutans, L. acidophilus. It has been established that in each milligram of dental plaque there might be approximately ten species of microorganisms *(Oilo & Bakken, 2015; Nabert-Georgi et al., 2018; Araujo, 2017)*. These microorganisms can colonize the oral cavity of a newborn in a matter of hours, and the physiological changes throughout its development, such as tooth eruption and tooth replacement, modify the microbial habitat *(Chan, 2010; Kunkel, 2019; González, 2017; Cruz et al., 2017)*.

Tooth surfaces do not detach their components, which makes them a stable anchoring surface for the development of biofilms *(Oilo & Bakken, 2015; Cruz et al., 2017)*. The formation of biofilm begins immediately after performing mouth cleaning, the saliva proteins cover the tooth surface and, then, bacteria attach to it through microfilaments in its cell walls. These microorganisms secrete glycoproteins, polysaccharides, nucleic acids and other substances to the extracellular matrix, which also contains glycoproteins and saliva nutrients, thus becoming an adhesion medium to biofilm. By increasing bacterial population, they are able to communicate with other bacteria secreting signaling molecules for forming colonies *(Oilo & Bakken, 2015; Nabert-Georgi et al., 2018; Cruz et al., 2017)*. Once the extracellular matrix is formed, it protects the internal bacteria from chemical agents, such as antibacterial mouthwash, which are lethal for bacteria that are floating freely on saliva *(Oilo & Bakken, 2015)*.

The aim of this article is to review the process of bacterial colonization of composite resins used with direct technique.

FORMATION OF BIOFILM

It starts with a process of progressive bacterial adhesion to the teeth surface or other structures in the oral cavity, over three stages:

a) Formation of biofilm on enamel: all the surfaces within the oral cavity are covered by a film of glycoproteins, components of the saliva and the gingival crevicular fluid, residues, bacterial products and host's cells; Van Der Waals, hydrophobic and electrostatic forces also intervene. This acts as a protection barrier that avoids tissue drying and enables bacterial adhesion *(Cruz et al., 2017; Cazzaniga et al., 2015; Sarduy & González, 2016)*.

b) Initial colonization: the first colonists are Gram-positive facultative microorganisms; they adhere to biofilm through adhesins that join receptors via strong covalent bonds that generate an irreversible union. Thus, mature biofilm is formed through the proliferation of adhered species, colonization and growth of new bacteria. A transition occurs from an aerobic to an anaerobic environment, the pioneer bacteria consume the oxygen, thus, favoring the proliferation of Gram-negative anaerobic bacteria *(Cruz et al., 2017; Cazzaniga et al., 2015; Sarduy & González, 2016)*.

c) Secondary colonization: the process of autogenic ecologic succession begins, with microorganisms that are better adapted to the acid environment, such as Prevotella intermedia and Fusobacterium nucleatum; then, bacteria are released, which can colonize new surfaces and repeat the process *(Cruz et al., 2017; Cazzaniga et al., 2015; Sarduy & González, 2016)*.

Characteristics such as thickness and composition of the biofilm depend on factors such as pH, nutrients, oxygen, time from the last cleaning and type of surface to which it is adhered, since the surfaces that present cracks, lines, abrasion defects and roughness, in the case of restorative materials, favor biofilm adhesion, being tooth resins more susceptible to bacterial colonization and adhesion *(Oilo & Bakken, 2015; Cazzaniga et al., 2015)*. The activity performed by biofilm is the demineralization of the hard surface of the tooth, causing dental caries. This is associated to the unbalance of oral microflora and an increase in acidogenic bacteria *(streptococci, lactobacilli, actinomyces and bifidobacteria)*. It has been established that S. mutans, a Gram-positive facultative anaerobic bacterium, is the main etiological agent of caries, since it has a high adhesion ability and acidogenicity in natural teeth, as well as in restorative materials *(Cruz et al., 2017; Menéndez, 2015; Lamont et al., 2015; Glauser et al., 2017)*.

COMPOSITE RESINS

Currently, the esthetic demand has led to the creation of materials that offer good esthetics and high durability. In restorative dentistry, composite resins are used in the treatment of prevalent diseases such as caries. Resins have been developed since 1962, the main components of these materials are: matrix, filling system, coupling agents, polymerization systems *(Cruz et al., 2017; Glauser et al., 2017; Vyavahare et al., 2014; Zeballos & Valdivieso, 2013)*. Dental adhesives are used for placing resins. These liquids contain low viscosity methacrylate, which spreads out over the dentin surface and becomes solid, allowing, on one side, adhesion to the resin composite and to dentin on the other side. Currently, there are two types of adhesive systems: the first system is total-etch and the second one is self-etch *(Zeballos & Valdivieso, 2013; Bourbia & Finer, 2018; Ionescu et al., 2017)*. In order to obtain a successful restoration, a protocol must be applied, which includes cleaning, isolation, complete removal of altered tissue, conformation of the cavity, protection of the dentin-pulp complex, adhesive system, stratification of composite, photopolymerization, occlusion control. A key step is performing proper finishing and polishing, thus ensuring a smooth surface that prevents bacterial adhesion *(Zeballos & Valdivieso, 2013; Ionescu et al., 2017)*.

BIOFILM TO RESIN ADHESION PROCESS

Resin-based composites contain ester bonds, which are vulnerable to the hydrolysis caused by the action of salivary esterase, provoking biodegradation and deterioration of the resin-based structure. The restorative material-tooth interface enables the entry of saliva and bacteria, thus contributing to recurrent caries, hypersensitivity and dental pulp inflammation. Finally, there is a release of degradation products, such as triethylene glycol methacrylic acid and bishydroxy-propoxy-phenyl-propane. This process generates more surface pores and defects, in comparison to metals, ceramics and enamel itself. The porosities act as incubation chambers that are perfect for certain microorganisms, which rapidly form the biofilm, full of acidic residues from bacteria, thus preventing its removal even more *(Oilo & Bakken, 2015; Zeballos & Valdivieso, 2013; Bourbia & Finer, 2018; Ionescu et al., 2017; Lamas-Lara et al., 2015)*. Microbial adhesion will depend on: composition and surface of the biomaterial, bacterial cellular surface, superficial load and hydrophobicity. Dental resins, when releasing ethylene glycol dimethacrylate, triethylene glycol dimethacrylate and dentin bonding agents *(hydroxyethyl methacrylate or ethylene glycol dimethacrylate)* stimulate the growth of cariogenic bacteria.

Currently, microhybrid and nanohybrid resins are used, which show advantages in polishing and shine due to the size of the inorganic filling particle, resulting in a less rough surface. Bacterial adhesion in microhybrid resins is 3,91 ± 0,52 colony-forming units *(CFU)* and in nanohybrid resins is 3,34 ± 0,74 CFU. Whereas composites with a particle size of 2.5 micrometers contained a greater volume of biofilms and enabled the adhesion of *S. mutans*, resins with particle size of 0.1 to 0.4 micrometers showed lower bacterial adhesion *(Moura et al., 2015; Motevasselian et al., 2017; Denson et al., 2018; Azam et al., 2015; 3M FiltekTM Z350XT, 2017)*.

When performing dental restoration with resinous materials, a perfect polishing and finishing technique must be used. This is a key process that prevents the formation of surface roughness and imperfections. A non-adequate polishing technique compromises the physical properties of the material and the restoration wears more rapidly *(Motevasselian et al., 2017; Denson et al., 2018)*. Some of the advantages of a good polishing are the reduction of periodontal disease, decrease of marginal discoloration, lower prevalence of secondary caries, esthetics, longer duration of the restoration, reduction of bacterial adhesion, creation of a clinically optimal surface. However, it has to be performed carefully, since the inadequate use of polishing materials might provoke greater surface roughness than before *(Motevasselian et al., 2017; Denson et al., 2018; Medeiros et al., 2016; Azam et al., 2015)*.

Nanotechnology is used to create resins that are smooth and show good physical properties. These resins show less polymerization contraction and improve the mechanical properties. By being polished and completely smooth, its longevity is prolonged and are highly esthetic; there are different materials that can be used for polishing: aluminum oxide discs, felt discs or pastes, silicon carbide brush *(Medeiros et al., 2016; Azam et al., 2015; Bezerra et al., 2016; Gharechahi et al., 2012; Bonilla et al., 2017)*.

NEW BIOMATERIALS THAT PREVENT BIOFILM FORMATION

Currently, several studies have been carried out on the development of resin-based restorative materials with antibacterial agents that are released gradually over time. Some antibacterial restorative composites contain components such as chlorhexidine, fluorides, silver nanoparticles or ursolic acid *(Neves et al., 2014; Neves et al., 2014)*.

Fluorine presents different properties: reduction of demineralization, interference in biofilm formation and inhibition of growth and metabolism of microorganisms. Some resins have been developed with strontium fluoride *(SrF_2)* filling, which is released gradually. The drawback is produced in the release stage by creating spaces in the matrix when fluoride leaves the material *(Kim et al., 2013; Subramani & Ahmed, 2018)*.

Chlorhexidine has been incorporated into resin-based restorative materials, it inhibits bacterial growth and its properties are lost over time due to the release of up to 50% of the bactericide agent within 14 days *(Kim et al., 2013; Subramani & Ahmed, 2018; Rutterman et al., 2015)*.

Silver nanoparticles present a wide spectrum of antimicrobial activity against oral streptococci, due to their affinity with molecular groups containing sulfur and phosphorus, which are present in the bacterial membrane. When these nanoparticles are inside of bacteria, they release silver ions that interrupt transmembrane electrons transfer and prevent DNA replication. The use of this material is a great alternative for the prevention of secondary caries; additionally, it is not toxic for human cells *(Kim et al., 2013; Subramani & Ahmed, 2018)*.

Ursolic acid is a compound that is found on vegetable species, it presents different biological anti-inflammatory and antimicrobial effects that inhibit the growth of Staphilococcus, Microsporum Lenosum and Cándida albicans, through the rupture of the cell wall, as well as the inhibition of protein synthesis and metabolism of nucleic acids *(Kim et al., 2013; Subramani & Ahmed, 2018; Frenzel et al., 2016)*. It presents an inhibitor effect on biofilm formation and *S. mutans* growth. Its drawbacks are discoloration of the biomaterial and gradual reduction of its effect.

DISCUSSION

Bacterial colonization and adhesion on hard tooth surfaces and dental composite resin restorations are processes that depend on the interactions of many factors such as: composition of restorative material and techniques used for polishing; as well as factors that are inherent to the person that presents restorations in the mouth, such as hygiene, diet and systemic factors.

Studies carried out by *Denson et. al. (2018)* and *Gharechani et.al. (2013)* proved the influence of nature, type of dental restoration material and surface roughness in bacterial colonization by cariogenic microorganisms. *Frenzel et.al. (2015)* structured nanohybrid composites with different microstructures *(flat, cubes, linear trapezoid structures, flat pyramids)*. They revealed a smaller amount of microorganisms in the flat samples, in turn, in linear trapezoid samples there was greater adherence. *Azam et. al. (2015)* revealed that particle size and wettability are also factors that modulate the degree of bacterial adhesion, since the bigger the particle size, the greater the adherence. Other factors such as availability of methyl radicals, type of bacterial strain and hydrophobicity of the resinous surface are also important; however, there no relation was found between surface roughness and plaque retention. *Motevasselian et.al. (2017)* compared the adherence of *S. mutans* on microhybrid resins, nanohybrid resins and dental amalgam, and did not find any big differences in the degree of bacterial colonization. Regarding polishing techniques, studies carried out by *Medeiros et.al. (2016)* and *Lamas et.al. (2015)* revealed greater CFU in Nealon technique *(19.6 ± 3.05)*, and 1.56± 0.62 in resins with laboratory polishing, which proved that the reduction in surface roughness accomplished through laboratory polishing presents lower bacterial adherence.

CONCLUSION

The degree of bacterial colonization depends on hygiene, polishing technique and composition of restorative material; the bigger the particle size, the greater the adhesion of bacterial plaque.

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