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Evaluation of the Degradation of a Resin Composed by the Metabolites Produced by *Streptococcus mutans*: *in vitro* Study

Avaliação da Degradação de uma Resina Composta pelos Metabólitos Produzidos por *Streptococcus mutans*: Estudo *in vitro*

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Abstract

Streptococcus mutans are microorganisms constituent of the oral biofilm, where their metabolites can promote superficial and microstructural alterations of the composites compromising their properties. The objective of this study was to evaluate, *in vitro*, the effect of the metabolites produced by this bacterium by means of 5% sucrose based culture medium on the surface and microstructure of a composite resin (Charisma®, Heraeus Kulzer®, Hanau, Germany). The samples were divided into four groups (N = 8): the external control group (G1), the internal control group immersed in BHI broth (G2), the G3 group was inoculated with *Streptococcus mutans* ATCC® 25175™ and in group G4 the same 5% sucrose associated microorganism was inserted. After the laboratory period, corresponding to 42 days, the samples were subjected to the surface analysis through the tests of microroughness, micrograph and surface microhardness obtained by means of *Vickers* assay. The results were submitted to One-Way Anova test, where it was verified that the G3 and G4 groups presented higher surface roughness and lower hardness when compared to the G1 and G2 control groups. It was concluded that the metabolites produced by *Streptococcus mutans* are capable of altering the surface and the microstructure of the composite evaluated in vitro, and in the mouth it could compromise the restorations longevity made with this type of material.

Keywords: *Streptococcus mutans*. Composite Resins. Bacterium.

Resumo

Os Streptococcus mutans são microrganismos constituintes do biofilme oral, onde seus metabólitos podem promover alterações superficiais e microestruturais dos compósitos comprometendo suas propriedades. O objetivo deste estudo foi avaliar, in vitro, o efeito dos metabólitos produzidos por essa bactéria por meio de um meio de cultivo à base de sacarose 5% sobre a superfície e microestrutura de uma resina composta. Foram confeccionados 32 corpos de prova em resina composta, os quais foram divididos em quatro grupos (N=8): o grupo controle externo (G1), grupo controle interno imerso em caldo BHI (G2), no grupo G3 foi inoculado o Streptococcus mutans ATCC® 25175™ e no grupo G4 foi inserido o mesmo microrganismo associado à sacarose 5%. Após o período laboratorial, correspondente a 42 dias, as amostras foram submetidas à análise da superfície através dos testes de microrrugosidade, micrografia e microdureza superficial obtida por meio de ensaio Vickers. Os resultados obtidos foram submetidos ao teste Anova One-Way, onde se verificou que os grupos G3 e G4 apresentaram maior rugosidade superficial e menor dureza quando comparados com os grupos controle G1 e G2. Concluiu-se que os metabólitos produzidos pelos Streptococcus mutans são capazes de alterar a superfície e a microestrutura do compósito avaliado in vitro e em boca, pode comprometer a longevidade das restaurações confeccionadas com este tipo de material.

Palavras-chave: *Streptococcus mutans. Resinas Compostas. Bactérias.*

1 Introduction

Composite resins have become the most widely used direct restorative materials in dental practice, due to the great demand of patients for aesthetic appearance. Among the restorative options, polymeric materials, such as composite resins, have undergone constant improvements in their physical, mechanical, aesthetic and manipulation properties.[1,2]

Despite their excellent characteristics, it has been observed in long-term studies that the main reasons for failure of this material are the restorations margins degradation, dental fractures and secondary caries.[3-5]

The dental biofilm is composed of a heterogeneous group of microorganisms and tends to stabilize over time. The teeth are colonized by bacteria, where *Streptococcus mutans* (*S. mutans*) is recognized as the main etiological agent of dental caries, acting on the sugars present in the diet (sucrose, glucose, fructose and lactose) by their energy metabolism, resulting in the production of acids, mainly lactic acid.[6-9] The metabolic activity of these microorganisms causes variations in the pH, being considered responsible for the biofilm installation, constituted by microbiota capable of surviving at acid pH (acid capacity). This is definitive for its pathogenicity and is responsible for the demineralization of enamel in the caries initial stage. This is due to the production of the acid resulting from the sucrose fermentation.[10,11]

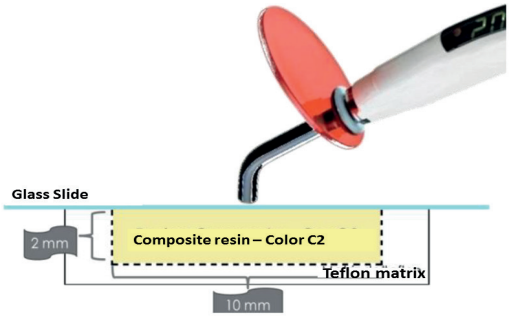
The dissemination of the use of the composite resin and the few existing studies on the influence of *S. mutans* on these materials were taken into account in order to develop this work,

whose objective was to evaluate the effect of metabolism of *Streptococcus mutans* in a 5% sucrose based culture medium on the surface of a composite resin used worldwide; and to verify whether these microorganisms have some implication in the microstructure of this resin, through micro-rugosity, micrograph and microhardness of Vickers test.

2 Material and Methods

Thirty-two specimens of composite resin, calculated according to sample Bolfarine and Bussab[12] with 2 mm thick and 10 mm in diameter, complying with ISO 4049: 2009, were used. For this purpose a micro-hybrid photopolymerizable composite resin (Charisma®, Heraeus Kulzer®, Hanau, Germany) was used. A Teflon® mold measuring 2 mm x 10 mm was used, where the resin was inserted by the single increment technique. With the aid of a Precision® microscope slide (26.0 x 76.0 mm and 1.0 mm thick), the resin was subjected to light pressure on the free face of this material to obtain uniformity (Figure 1).

Figure 1 - Preparation of test specimens



Source: Authors

The specimens were photoactivated for 40 seconds on each face using an Optilight Max (LED) light emitting diode (Gnatus®, Ribeirão Preto, São Paulo, Brazil). At wavelengths between 420nm - 480nm and light output of 1200 mW / cm2 ± 200 mW / cm2. The specimens were fixed in wires prior to sterilization in ethylene oxide

Streptococcus mutans were reproduced from the ATCC® 25175 ™ sample. The 32-test specimens were randomized into four groups (N = 8). One group was maintained only at room temperature in a dry environment, being considered as an external control group (G1). The other specimens were distributed in three experimental groups. The group considered internal control (G2) was submerged in test tubes containing BHI broth. The G3 group had BHI broth in association with *Streptococcus mutans* and the G4 group, BHI broth, *Streptococcus mutans* and 5% sucrose.

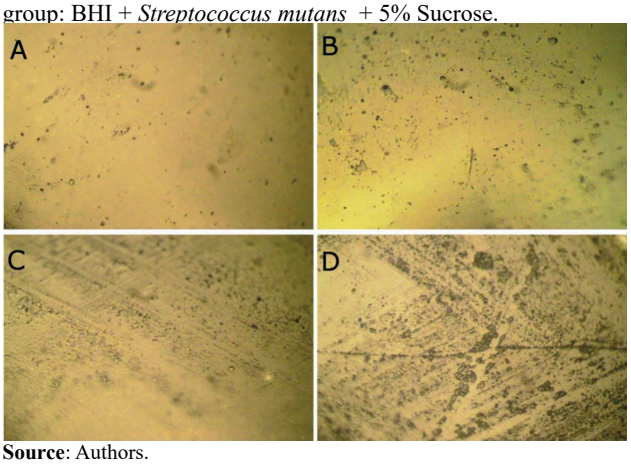
The exchanges of the culture medium occurred weekly, following the precepts of the aseptic chain occurred during a 42-day period. The pH was measured with pH Indicator trips MERCK®. The pH with sucrose-free *Streptococcus mutans* (Group G3) was 5.5 and the Ph from the G4 (with sucrose) group reached 4.5. After the exchanges, the test tubes were kept on the laboratory benches of microbiology at room temperature and properly identified. At this time the specimens were removed from the test tubes and washed in sterile distilled water and dried with sterile absorbent paper. After the laboratorial stage, all the specimens were subjected to a qualitative analysis by means of micrographs, and quantitative tests of micro-roughness (TIME TR200 RoughnessTester) and Vickres microhardness (HMV - Micro Hardness Tester). Five measurements were performed, all by a single blind calibrated examiner for the samples.

The data obtained were compared by the ANOVA test with significance of 0.05, followed by the Tukey test carried out with the aid of the Past.exe software, using the Microsoft Excel 2010 program, with significance of 5%.

3 Results and Discussion

Based on the methodology described above, the qualitative analysis of the specimens obtained through micrographs is available in Figure 2.

Figure 2 – (A) G1 group: external control. (B) G2 group: internal control. (C) G3 group: BHI + *Streptococcus mutans*. (D) G4 group: BHI + *Streptococcus mutans* + 5% Sucrose.



Source: Authors.

About quantitative analyses, obtained by surface roughness and microhardness by Vickers tests, the average values of each test specimen were obtained and the same was subjected to ANOVA test (Tables 1 and 2). It was found that there was a statistically significant difference among all groups in the surface roughness test (p = 0.00000000648) and Vickers test (0.0000689).

Table 1 - Anova: single factor - Surface roughness (µm)

Variation Source	SQ	gl	MQ	F	P-value	Critical F
Among all groups	0.42120375	3	0.14040125	30.16190628	0.00000000648*	2.946685
Within the groups	0.13033775	28	0.00465492			
Total	0.5515415	31				

* Significant difference (p<0.05).

Source: Authors.

Table 2 - Anova: single factor – Vickers test (HV)

Variation Source	SQ	gl	MQ	F	P-value	Critical F
Among all groups	2183.983	3	727.994333	10.80468	0.0000689*	2.946685
Within the groups	1886.5752	28	67.3776857			
Total	4070.5582	31				

* Significant difference (p<0.05).
Source: Authors.

The Tukey test was then performed to identify which group was different from the others (Tables 3 and 4). Groups G3 and G4 presented values <0.05, therefore they were statistically different from Groups G1 and G2.

Table 3 - Tukey's Test - Surface Roughness

	G1	G2	G3	G4
G1		0.9943	0.0007325*	0.0001645*
G2	0.3576		0.001301*	0.0001645*
G3	6.358	6.001		0.005394*
G4	11.55	11.19	5.187	

* Significant difference (p<0.05).
Source: Authors.

Table 4 - Tukey's Test – Vickers test

	G1	G2	G3	G4
G1		0.4463	0.01007*	0.0002113*
G2	2.133		0.2479	0.003446*
G3	4.831	2.698		0.2352
G4	7.574	5.441	2.743	

* Significant difference (p<0.05).
Source: Authors.

Laboratory tests do not accurately reproduce in vivo findings, but they represent a respectable parameter of analysis, since if the material exhibits satisfactory in vitro performance, it will likely result in good clinical performance.[1-8,11] With the popularization of the use of composite resins in dentistry, a large number of studies were carried out to identify the degradation of this material when subjected to various types of damages, such as: masticatory and occlusal forces, food with acidic pH, among others.[1-9,14-16] However, the potential effect of bacterial degradation activity on composite resins has not yet been extensively explored.[15]

Biofilm formation occurs not only in hard dental tissues, but also affects the surfaces of restorative materials. The susceptibility of restorative materials to adhere the microorganisms is analyzed as extremely important to promote their longevity in the oral cavity.[4,10]

Exposure of restorative materials to lactic acid can reduce the hardness and increase the surface roughness, favoring even more the biofilm accumulation [3,17] which was evidenced in this in vitro study, where the group denominated G4 by having the energetic potentiating factor of sucrose 5%, promoted a greater drop in pH, directly influencing the reduction of hardness and increase in surface roughness of the composite resin in question.

The studies of Pala et al.[18] and Koh et al.[19] assure that the composite resins present an organic matrix and inorganic particles in their formulation, in which they provide different hardnesses, suffering different wear when subjected to occlusal forces. Because of its low hardness, the organic matrix degrades faster and exposes the inorganic loads that are then dislodged by friction. Therefore, the larger the fillers of charge, the greater the roughness left by the inorganic matrix. According to the manufacturer's information, CHARISMA® is produced on the basis of BIS-GMA and contains 58% of its volume of charge particles. The barium glass particles have an average size of 0.7μm and a maximum size of less than 2μm. The biofilm containing *S. mutans* grown on the surface of resin-based composites increases the surface roughness of the material as observed in this study and in the study by Cazzaniga et al.[17] The increase in surface roughness facilitates adhesion and formation of bacterial biofilm in resin-based composites, making the restoration susceptible to failure and interfering with its durability. [18] Then, the surface roughness is directly proportional to the biofilm accumulation, where in theory the higher roughness of the G4 group and the change in the material hardness of the same group were given to the cohesive biofilm formation on the composite resin surface capable of releasing acids on the composite, resulting in the reduction of its hardness.The acids produced by the cariogenic biofilm, acid diet and salivary enzymes may also lead to a reduction of hardness and increase in the surface roughness of resinous materials. [20,21] In the tests of roughness and microhardness of the present study it is possible to verify that the specimens that had contact with the *S. mutans* associated or not with the sucrose, presented worse performance compared to the control groups, being in agreement with the literature. [17-21]

A cariogenic diet may lead to loss of properties of resinous restorative materials. Substances with pH between 5.0 and 7.0, lead to the loss of resinous materials by similar disintegrations, at a pH lower than this, the loss is even more pronounced, causing changes in the surface integrity and cracks formation in the composites. This occurs because the acid degrades the resin matrix, exposing particles of inorganic filler, there being interaction between solvent-polymer. Polymers in contact with acidic subtances lose their secondary bonds among the macromolecules, causing the material to decrease its hardness.[22,23]The loss of the physicochemical properties of the composite resin evaluated was evidenced in groups G3 and

G4 of this study, and there was a greater degradation in the latter group, considering the potentiation of the degradative effect of the *S. Mutans* in association with sucrose, causing the material to lose hardness and increase its surface roughness.

This leads us to assume that the metabolites produced by this microorganism in the mouth through the reduction of hardness and increased surface roughness can directly interfere in the direct restorations longevity in composite resin, leading to the occurrence of fractures, restoration margins degradation, secondary caries and color change. The limitations of this study refer to the use of only one trademark and one type of composite resin. It is therefore suggested that more studies like this be carried out, evaluating and comparing other commercial brands and other types of composite resins available in the market.

4 Conclusions

In Conclusion, the metabolites produced by *S. Mutans* in vitro increased the roughness and decreased the hardness of the composite resin (Charisma® - Heraeus Kulzer®, Hanau, Germany) and when they received the energetic stimulus of 5% sucrose, these findings were even more expressive.

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