IN VITRO EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF DENTAL MATERIALS AGAINST *Streptococcus mutans*

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ABSTRACT

Secondary caries is related to the colonization of the tooth restoration interface by cariogenic microorganism. Believed that the use of materials with potential antimicrobial activity, either as protection of the dentin-pulp complex or as restoration, may have a role in preventing secondary caries. To verify this, we analyzed the following materials: acrylic resin; glass-ionomer, Maxxion-R; tooth sealant, Ultra Seal XT- Ultradent; fluid resin, Perma Flo – Ultradent; ionomer sealant, Clinpro XT Varnish – 3M; and adhesive system, PQ1 – Ultradent. The agar diffusion test performed to determine the bacterial growth inhibition of *Streptococcus mutans*. The materials were prepared according to the manufacture´s recommendations under aseptic conditions, and placed in Petri dishes containing solid BHI medium with 0.2 U/L bacitracin (selective medium for *S. mutans*), prepared in triplicate. The plates incubated at 37ºC with 5% CO_2 and observed daily for 7 days. The antimicrobial activity of these materials obtained by the ratio between the diameter of the specimen and the diameter of the halo of growth inhibition formed around it. As expected, the acrylic resin (negative control) showed no antimicrobial activity, while the glass-ionomer (positive control) showed inhibitory activity. Between the tested materials, only the ionomer sealant and adhesive system showed significant antimicrobial activity, compared to controls.

KEYWORDS: Products with Antimicrobial Action, Dental Materials, Dental Caries, *Streptococcus mutans*.

1. INTRODUCTION

Due to its high prevalence, dental caries is still considered a public health problem, especially in lower socioeconomic status communities, where the high consumption of sugar and other fermentable carbohydrates may interfere with the equilibrium of the host’s oral microbiota[11,14-22].

Regarding the clinical longevity of the dental restorative procedures, secondary caries is a relevant factor in restoration replacement[s7,17-21], particularly due to the interface created between the tooth and the restoration after the cavity preparation, that can favor the marginal microinfiltration and its colonization by potentially cariogenic microorganisms. This event becomes more likely with the polymerization contraction of improper polymeric restorative materials[6].

In addition to this, in order to minimize the retention of oral plaque and, consequently, prevent the formation of carious lesions around restorations, one should also consider factors such as surface smoothness after finishing and polishing the restoration, and the antimicrobial action of the dental materials. In patients more susceptible to caries, the higher the surface roughness of restorations, the higher the colonization by potentially cariogenic microorganisms, increasing the likelihood of secondary caries[23]. However, the metabolism of the microorganisms that colonize restorations can be minimized with restorative materials bearing antimicrobial properties[9,26-33.]

The aim of this study is to evaluate the *in vitro* antimicrobial properties of different dental restoration materials against *Streptococcus mutans*, one of the major causative agent of dental caries.

2. MATERIAL AND METHODS

To test the antimicrobial activity of six dental materials we used a standard strain of *Streptococcus mutans* (UA159). The cariogenic strain UA159 we used in the genome sequencing of *S. mutans* and kindly donated by Professor Rita de Cássia Café (Institute of Biological Sciences of the University of São Paulo).

The bacteria maintained in Petri plates with solid brain heart infusion (BHI - DFICO) and kept under refrigeration. For the experiments, we removed a small amount of culture from the plates with a platinum inoculation loop, diluted in 5 mL of liquid BHI and incubated for 16 hours at 37ºC and 5% CO_2 and no agitation.

The following materials were analyzed: acrylic resin, JET-Classic; conventional glass ionomer cement, Maxxion R – FGM; mono component adhesive system, Pq1 – Ultradent; fluid composite resin, Perma-Flo – Ultradent; resinous sealant for pits and fissures, Ultraseal – Ultradent; and ionomeric sealant Clinpro Varnish XT - 3M ESPE. Disc-shaped specimens were prepared with the mentioned materials measuring 6 mm of diameter and 2 mm thickness (Figure 1). All materials were handled in aseptic conditions, following the manufacturer's instructions.

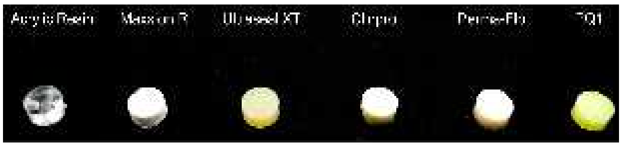

Figure 1. Specimens prepared for the agar diffusion test. Dimensions: 6 mm of diameter and 2 mm thickness. From left to right: acrylic resin, conventional glass ionomer cement, monocomponent adhesive system, fluid compound resin, resinous sealant of pits and fissures and ionomer sealant.

We inserted all specimens into a metallic matrix with same dimensions as mentioned above (Figure 2), to polymerization. For the acrylic resin and ionomer cement, we waited for the chemical cure; meanwhile, polymerized physically via photopolymerizer the other four materials.

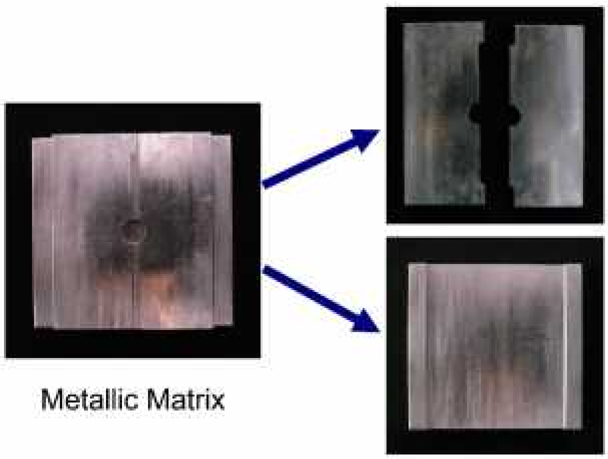

Figure 2. Matrix used for the specimens.

In order to evaluate the ability of the analyzed materials to release antimicrobial compounds into the medium, we conducted an agar diffusion method. The specimens were placed on the surface of solid BHI medium (10 mL) added with 0.2 U/mL Bacitracin (Sigma Aldrich), in Petri plates, with 100 µL of *S. mutans* (obtained from the cultures described above). Finally, the plates were incubated at 37ºC and 5% CO_2 and observed daily for up to 7 days. The antimicrobial activity was measured as the diameter of the halo of growth inhibition around the specimens. For a better visualization of the results, the plates were stained with crystal violet and photographed. The antimicrobial activity of the different materials was calculated as an inhibition zone (IZ), given as the ratio between the growth inhibition halo diameter and the specimen diameter. The acrylic resin and the glass ionomer were used as negative and positive controls, respectively. We conducted the experiment in triplicate, with two repetitions. We obtained the averages and standard deviations of the inhibition zones for each material and the statistical significance of the results were assessed with Student *t* test conducted on Excel (Microsoft).

3. RESULTS

As expected, the acrylic resin (negative control) did not show any antimicrobial activity, while the glass ionomer cement (positive control) showed an inhibition halo, proving the efficacy of the method.

According to Figure 3, both the ionomer sealant (Clinpro XT) and the dental adhesive (Pq1) showed antimicrobial activities against *S. mutans* similar to the positive control. On the other hand, the resinous sealant (Ultraseal XT) and the fluid resin (Perma-Flo) showed no statistically significant antimicrobial activity.

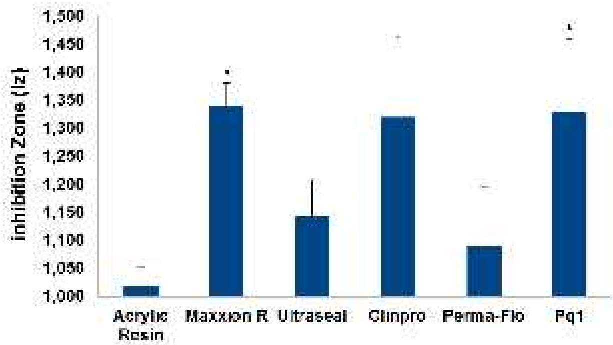

Figure 3. Graph with the averaged Iz for each material. The experiment was conducted in triplicate, with two repetitions. *Statistically different from the acrylic resin (p<0,05).

4. DISCUSSION

Given the high frequency of restoration replacements due to secondary carious lesions[5-25], some features are recommended for adhesive restorative materials such as low polymerization contraction, linear thermal expansion coefficient, physical mechanical properties similar to those of the dental substrate, and antimicrobial activity, capable of protecting the dental substrates from the adverse effects of a possible marginal microinfiltration. In this study, the activity of six dental restoration materials against a strain of *S. mutans* was evaluated in BHI agar medium. Among these, the acrylic resin (JET-Classic) was used as negative control due to the lack of antimicrobial compounds in its composition[20-27]. The lack of antimicrobial activity in a compound resin can be evaluated by a test proposed by Maara *et al.* (2012)[16]. In their study, the test was conducted with and without PTBAEMA against *Staphylococcus aureus*, *Streptococcus mutans*, and *Candida albicans* and showed no antimicrobial activity for the resin alone. On the other hand, the conventional glass ionomer cement (Maxxion, FGM) was used as positive control given its capacity to release fluoride and, therefore, to inhibit bacterial growth and to alter the process of demineralization and remineralization of the carious lesion[36].

Due to the range of clinical indications, these two materials were compared to a mono component adhesive system (Pq1, Ultradent); a fluid composite resin (Perma-Flo, Ultradent); a resinous sealant for pits and fissures (Ultraseal, Ultradent); and an ionomeric sealant (Clinpro Varnish XT, 3M ESPE). The microorganism was chosen considering its relation to the caries etiology and its availability in laboratorial research[1,15].

Our results showed that both the ionomer sealant and the mono component adhesive system showed antimicrobial activity similar to the conventional glass ionomer cement. Regarding the latter, the antimicrobial activity may not be attributed only to the fluoride release, which is influenced by the curation speed, but also to the presence of particles of Calcium, Aluminum, and Silicate[36]. The fluoride release process is directly affected by the composition of the material, its storage, the powder/liquid ratio, and the handling method, the pH of the medium, the material porosity after curation, and the type of material used on the surface protection[24]. Contrarily to what is recommended by the manufacturer, we did not use any surface protection material in order to avoid any changes in the concentration of fluoride released during the evaluation period.

When compared to the modified glass ionomer cement added with resinous monomer, the conventional cements are more soluble, more porous, present more irregular particles and higher powder/liquid ratio and, therefore, release larger concentrations of fluoride[12-29]. However, our results showed a similar antimicrobial behavior between the Maxxion R (FGM) cement and the ionomer sealant Clinpro XT Varnish (3M ESPE), a glass ionomer modified by resin indicated to the treatment of dentin hypersensitivity and sealing of pits and fissures. Since it releases fluoride, calcium and phosphate ions, it has antimicrobial properties and the capability to increase the dental surface resistance against the corrosive actions of acids. It is also indicated for application around the dental brackets to control the demineralization of the tooth enamel[2-10].

The antimicrobial property of the mono component adhesive system may be related to its low pH, a common feature for simplified adhesive systems[8]. In addition, this material presents the resinous monomer hydroxyethil methacrylate (HEMA) in its composition that, similarly to the triethylene glycol dimethacrylate (TEGMA), does confer antimicrobial activity to the resinous materials. This property arises in adhesive systems with molecules of glutaraldehyde, fluoride and/ or 12-methacryloyloxy dodecyl pyridinium (MDPB), and methyl methacrylate ammonium chloride (DMAE-CB) in its composition[30,34].

According to the results shown, the acrylic resin, the resinous sealant for pits and fissures and the fluid resin lacked antimicrobial activity. Therefore, pathogenic microorganisms such as Candida albicans and *S. mutans* can colonize the ridges and pores on their surface[20,27,31,32]. Aiming at reducing the microbial colonization on the surface of the acrylic resin, Regis *et al.* (2011)[28] incorporated MDPB to the material. However, this antimicrobial activity presented a defined lifespan and interfered with the mechanical properties of the resin28. Recent efforts to incorporate silver particles to these materials have shown promising results regarding antimicrobial activity.

The resinous sealant and the fluid resin did not show significant activity in comparison to the negative control, even with the addition of sodium monofluorphosphate to their composition. Regarding the amount of fluoride in the resinous monomer, although a smaller quantity of the ion reduces the antimicrobial activity of the resin, it also renders the material more translucent, a desirable feature for restorative compounds[18,35].

If correctly applied, ensuring the prevention of microinfiltration, the lack of antimicrobial activity of the sealant of pits and fissures will not affect its clinical indication and its preventive action against the colonization by cariogenic microorganisms in areas of fissures and pits, of difficult hygienization[3]. Another point worth observing during the sealant application is the relation between its longevity and efficacy. In a systematic review, Kühnisch *et al*. (2012)[13] observed that the sealants retention time is five years and, regardless of its lack of antimicrobial activity, it should be indicated for the mechanical control of the bacterial plaque on the retentive surfaces.

5. CONCLUSION

According to the methodology used in this study, we can conclude that the sealant for pits and fissures and the fluid compound resin did not present antimicrobial activity. On the other hand, the ionomer sealant modified by photopolymerizable resin and the adhesive system present activity against *Streptococcus mutans*. Thus, this study suggests that the ionomer sealant and the dental adhesive can be used to prevent secondary carious lesions due to their antimicrobial activity.

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