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Antibacterial activity of four glass ionomer cements used in atraumatic restorative treatment

Renata Cristiane da Silva · Angela Cristina Cilense Zuanon · Denise Madalena Palomari Spolidorio · Juliana Alvares Duarte Bonini Campos

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Abstract The in vitro antibacterial activity of four glass ionomer cements (Fuji IX, Ketac Molar, Vidrion R and Vitromolar) indicated for Atraumatic Restorative Treatment (ART) was studied against strains of bacteria involved in the development of oral diseases, *Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilus* and *Actinomyces viscosus.* The agar plate diffusion test was used for the cultures, which included chlorhexidine as a positive control. The results demonstrated that all the cements evaluated presented antibacterial activity. Based on the results of this study, it can be concluded that Fuji IX and Ketac Molar presented the most effective antibacterial activity considering the ART approach.

Introduction

Dental caries can be considered one of the most important pathological processes in humans. According to World Health Organization [1], three quarters of the worlds population suffer from untreated caries. Acidogenic bacteria are the first microorganisms to contribute to the evolution of dental caries [2].

R. C. da Silva · A. C. C. Zuanon (✉) ·
J. A. D. B. Campos
Department of Pediatric Dentistry and Orthodontics, Araraquara Dental School-UNESP-São Paulo State University, Rua Humaitá, 1680, 14801-903 Araraquara, SP, Brazil
e-mail: re_cri@yahoo.com.br

D. M. P. Spolidorio
Department of Physiology and Pathology, Araraquara Dental School, São Paulo State University, UNESP, SP, Brazil

The Atraumatic Restorative Treatment (ART) approach was developed to treat dental caries in those countries where human and physical resources are not available. The technique consists in minimal removal of carious tooth structure and then restoring the cavity and sealing any adjacent enamel fissure with conventional glass ionomer cement [3, 4].

Incomplete removal of caries has been advocated in techniques such as ART where the removal of soft demineralized carious dentine allows tooth reparation. In these procedures, glass ionomer cements are used to reduce the viability of residual bacteria [5], preventing the occurrence of secondary caries [6].

Glass ionomer cements exhibit biocompatibility with dental pulp, chemical adhesion to tooth enamel and dentin structure, release of fluoride ions among others. Fluoride is considered to be an important factor in the anti-cariogenic property of these materials [7–9].

As growth inhibitory effects of some restorative materials are considered beneficial in preventing bacterial colonization, the purpose of this study was to investigate the growth inhibitory effect of Fuji IX, Ketac Molar, Vidrion R and Vitromolar against *Streptoccocus mutans, Streptococcus sobrinus, Lactobacillus acidophilus* and *Actinomyces viscosus.*

Materials and method

The dental materials evaluated in this study are shown in Table 1. The anti-bacterial activity of each material was evaluated against *Streptococcus mutans* (ATCC#25175), *Streptococcus sobrinus* (ATCC#27607), *Lactobacillus acidophilus* (ATCC#IAL-523) and *Actinomyces viscosus* (T14 V#IAL.5) using the agar plate diffusion test [10].

Table 1 Materials used in the study

Materials	Classification	Composition	Lot
Fuji IX			
GC Corporation (Tokio, Japan)	Conventional glass Ionomer	[a]Alumino-flurosilicate glass [b]Water, polyacrilic acid, polybasic carboxylic acid	0001251
Ketac molar			
ESPE Dental AG (Seefeld, Germany)	Conventional glass ionomer	[a]Al-Ca-La flurosilicate glass, 5% copolymer acid (acrylic and maleic acid) [b]Polyalkenoic acid, tartaric acid, water	119862[a]/119169[b]
Vidrion R			
SS White Artigos Dentários Ltda (RJ, Brazil)	Conventional glass ionomer	[a]Na-Ca-Al-flurosilicate-Ba sulfate, acrylic acid [b]Tartaric acid, water	00[A] [a]/00U[b]
Vitromolar			
DFL Indústria e Comércio Ltda (RJ, Brazil)	Conventional glass ionomer	[a]Ba-Al silicate, dehydrated polyacrylic acid and iron oxide [b]Polyacrylic acid, tartaric acid and distilled water	02010440

[a] powder, [b] liquid

Indicator strains were grown in Brain Heart Infusion broth (BHI™, Difco Laboratories, Detroit, MI) for 48 h at 37 °C, according to the physiological characteristics of each microorganism. The resultant bacteria were again placed in 5 mL BHI for 24 h at 37 °C to form a suspension corresponding to 10^6 CFU/mL (inoculum).

In each sterilized Petri dish (20 × 100 mm), a base layer containing 15 mL of BHI agar mixed with 300 µl of each inoculum was prepared. After solidification of the culture medium, five wells measuring 4 mm in diameter were made in each plate and completely filled with one of the testing materials listed in Table 1 (Fig. 1). Ten wells were filled with each material and bacteria strain. All materials were handled under aseptic conditions according to the manufacturer's instructions. A half a microliter of aqueous 0.2% chlorexidine digluconate was applied on a 4 mm diameter sterile filter paper discs (n = 10), which were the control group. The plates were kept for 2 h at room temperature for diffusion of the materials. After this time, they were incubated at 37 °C for 48 h.

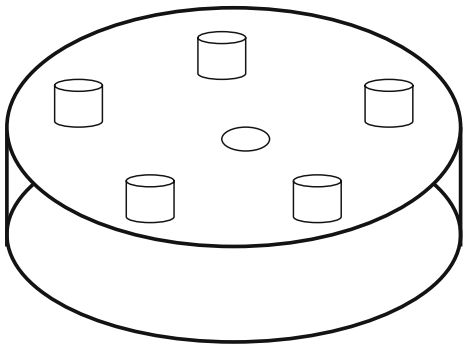

Fig. 1 Layout of petri dish for agar diffusion test

Zones of bacterial growth inhibition were recorded in millimeters (mm) using a digital caliper (Mitutoyo, SP, Brazil). Measurements were taken at the greatest distance between two points at the outer limit of the inhibition halo formed around the well. Antibacterial tests were repeated three times to confirm the homogeneity of the results. The mean diameter of inhibition zone values for each material was used for statistical analysis by means of Kruskall-Wallis non-parametric tests to compare inhibition zones of the materials against each bacteria strain at a significance level of 5%. Complementary Mann-Whitney tests (Wilcoxon rank-sum tests) were performed to identify the difference by pairing the materials.

Results

The mean values and standard deviations of the inhibition zones for each material according to the bacteria strain are shown in Table 2. Antibacterial activity was only considered to have occurred when a true inhibition zone was present, whether associated or not with the diffusion zone.

Discussion

Atraumatic restorative treatment is an approach based on caries removal using hand instruments and restoring the cavity with an adhesive material, usually glass ionomer cement. This dental material presents favorable and important properties such as biocompatibility to dental pulp, capacity of bonding chemically to enamel and dentin and releasing fluoride, which can play an important role in inhibiting the growth of bacteria and caries progression [2, 4, 11].

Table 2 Mean of diameters of inhibition zones (mm) evaluated on inoculated agar plates after 48 h

Bacteria	Materials				
	Fuji IX	Ketac molar	Vidrion R	Vitromolar	Chlorexidine
S. mutans	12.4[a]	10.0[b]	11.4[a]	9.6[b]	22.5[c]
S. sobrinus	11.4[a]	10.0[a,c]	7.0[b]	9.0[c]	25.5[d]
L. acidophilus	11.8[a]	12.6[a]	9.0[b]	9.2[b]	22.5[c]
A. viscosus	6.6[a]	8.8[b,c]	6.4[a]	7.2[a,c]	28.75[d]

Means followed by the same letter are not different according to the Mann-Whitney test ($P > 0.05$)

Different in vitro methods have been used to study antibacterial activity of dental materials. Boeckh et al. [12] throughout their experiments using strains of *S. mutans*, showed the important role of this microorganism in caries etiology. However, they suggested the need for further studies, which would employ other oral microorganisms including *Streptococus sp., Lactobacillus sp., Actinomyces sp* and Gram-negative anaerobes. In this study, strains of *S. mutans, S. sobrinus, A. viscosus, L. acidophilus* were used, which present important role in oral diseases.

According to other studies, the present methodology used agar plate diffusion to verify the inhibition zone of the materials evaluated. According to the results, all glass ionomer cements evaluated inhibited bacterial growth, but with differences according to material and microorganism. Fuji IX and Vidrion R demonstrated the greatest inhibitory activity for *Streptococcus mutans* while Ketac Molar and Fuji IX were statistically effective against *Streptococcus sobrinus* and *Lactobacillus acidophilus.* In addition, Ketac molar also demonstrated the greatest inhibiting halo against *Actinomyces viscosus* in contrast to the other glass ionomer cements.

Antibacterial activity of such materials has been related to either initial setting low pH, fluoride release or other chemical components present in the powder of these materials [13].

Glass ionomer cements release various ions, of which fluoride has expressed antibacterial properties and presented potential of preventing caries. The amount of fluoride release has been related to the composition and cure reaction of the material [2, 12].

High strength conventional glass ionomer cements Fuji IX and Ketac Molar present high powder/liquid ratios that improve their mechanical properties for restorations in posterior teeth. However, this can result in decreased solubility and fluoride liberation [8, 9].

Considering the results in the present study, the greatest inhibitory halos were demonstrated by high-strength conventional glass ionomer cements Fuji IX and Ketac Molar. These materials are specifically marked for the ART approach and release less amounts of fluoride ions than those conventional and resin modified glass ionomer cement [8]. Thus, we can suggest that the antibacterial activity may also be related to other released ions.

Some authors suggested that the release of metallic ions and the low initial pH of setting reaction are more significant than fluoride ion release for any antibacterial properties that may be present [13]. However, the effects of short and long term release of other elements from glass ionomer cement such as aluminum, strontium, calcium, silicon and phosphorus remain to be determined [8, 13, 14].

In spite of the antibacterial activity of the glass ionomer cements evaluated, the inhibiting zone was smaller than chlorhexidine, which produced an average inhibition zone of 28.75 mm. The use of chlorhexidine mouth rinses to control dental plaque and gingivits has been well established. Microbial sampling has shown a reduction in counts of both aerobic and anaerobic bacteria ranging from 54% to 97% through 6 months of use [15]. Chlorhexidine was chosen as the positive control because of its widespread clinical use, plus it serves as a common point of reference for comparisons with others studies [2].

The present study suggests that mechanisms of glass ionomer antibacterial effect must be elucidated, the release of others ions must be investigated and their exact role explained. Another important question to be answered is if this antibacterial activity is sufficient to stop bacterial metabolism or to destroy such microorganisms.

Antibacterial activity of a restorative material is a significant property. Not only can it play a decisive role in preventing secondary decay, but also interfere in microorganism metabolism. In addition, it may inhibit bacterial growth or even stop it. Hence, studies concerning the incorporation of others substances that exhibit antibacterial activity in the glass ionomer cement composition and deeper investigation about the chemical role of some ions released by this material.

Conclusion

Based on the results of the present study, it can be concluded that all glass ionomer cements evaluated demonstrated antibacterial activity with differences according to material and microorganism. Furthermore, the glass ionomer cements Fuji IX and Ketac Molar showed the best antibacterial activity when considering the ART approach.

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