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Inhibitory effects of a cured antibacterial bonding system on viability and metabolic activity of oral bacteria

Éfani C.F. Banzi[a], *Ana R. Costa*[b], *Regina M. Puppin-Rontani*[c,*], *Jegdish Babu*[d], *Franklin García-Godoy*[e]

[a] *Pediatric Dentistry Division, Piracicaba Dental School, University of Campinas – UNICAMP, Piracicaba, São Paulo, Brazil*

[b] *Dental Materials Division, Piracicaba Dental School, University of Campinas – UNICAMP, Piracicaba, São Paulo, Brazil*

[c] *Pediatric Dentistry and Dental Materials Division, Piracicaba Dental School, University of Campinas – UNICAMP, Piracicaba, São Paulo, Brazil*

[d] *Department of Bioscience Research, University of Tennessee Health Science Center, College of Dentistry, Memphis, TN, USA*

[e] *Department of Bioscience Research, Bioscience Research Center, University of Tennessee Health Science Center, College of Dentistry, Memphis, TN, USA*

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ABSTRACT

Objectives. To evaluate the antimicrobial efficacy of Clearfil SE Protect (CP) and Clearfil SE Bond (CB) after curing and rinsed against five individual oral microorganisms as well as a mixture of bacterial culture prepared from the selected test organisms.

Methods. Bacterial suspensions were prepared from single species of *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus gordonii*, *Actinomyces viscosus* and *Lactobacillus lactis*, as well as mixed bacterial suspensions from these organisms. Dentin bonding system discs (6 mm × 2 mm) were prepared, cured, washed and placed on the bacterial suspension of single species or multispecies bacteria for 15, 30 and 60 min. MTT, Live/Dead bacterial viability (antibacterial effect), and XTT (metabolic activity) assays were used to test the two dentin system's antibacterial effect. All assays were done in triplicates and each experiment repeated at least three times. Data were submitted to ANOVA and Scheffe's *f*-test (5%).

Results. Greater than 40% bacteria killing was seen within 15 min, and the killing progressed with increasing time of incubation with CP discs. However, a longer (60 min) period of incubation was required by CP to achieve similar antimicrobial effect against mixed bacterial suspension. CB had no significant effect on the viability or metabolic activity of the

* *Corresponding author at*: Pediatric Dentistry and Dental Materials Division, Piracicaba Dental School, University of Campinas – UNICAMP, Av. Limeira, 901, 13414-903 Piracicaba, São Paulo, Brazil. Tel.: +55 19 21065286; fax: +55 19 21065218.
E-mail addresses: rmpuppin@fop.unicamp.br, rmpuppin@gmail.com (R.M. Puppin-Rontani).
URL: http://www.fop.unicamp.br (R.M. Puppin-Rontani).

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test microorganisms when compared to the control bacterial culture. CP was significantly effective in reducing the viability and metabolic activity of the test organisms.

Significance. The results demonstrated the antimicrobial efficacy of CP both on single and multispecies bacterial culture. CP may be beneficial in reducing bacterial infections in cavity preparations in clinical dentistry.

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1. Introduction

Adhesive restoration is the most common treatment in the daily practice of dentistry, especially in minimal intervention procedures [1]. However, partial caries removal is based on established clinical criteria (darker and harder dentin), and it is not possible to confirm the absence of viable cariogenic bacteria in the remaining dentin when using a minimally invasive procedure.

Antibacterial treatment of the dentin possibly suppresses the growth of bacteria under existing restorations and thus minimizes the risk of recurrent caries and damage to the pulp [2]. In order to increase the success rate of the restorative procedures, many studies have demonstrated the antibacterial benefits of incorporating antimicrobials into the primer of dentin bonding system against bacteria associated with dental caries [3,4].

Studies with dentin bonding systems have pursued improvements and advancements focusing on the development of an antibacterial monomer having antibacterial properties [4,5] in addition to being able to increase bond strength [6], which would provide reliable results [7] in treating dental caries.

To achieve these goals, antibacterial agents have been added to the monomers in dentin bonding systems. The monomer, methacryloyloxydodecylpyridinium bromide (MDPB), is a quaternary ammonium compound, an antibacterial agent, with a methacryloyl group [8]. This antibacterial agent is covalently bonded to the polymer matrix through copolymerization of MDPB with other monomers during curing. The antibacterial effects of immediately polymerized dentin bonding are beneficial by eradicating the residual bacteria in the oral cavity [5]; however, this property seems to be diminished after the curing process [9]. Contact time is a preponderant factor when using antimicrobial agents. Long-lasting antibacterial activity of the polymerized dentin bonding system may be effective in inactivating bacteria that invade the tooth-dentin bonding interface by microleakage [10].

Previous studies [3,7,11] showed strong antibacterial activity of MDPB against *S. mutans* and also against other oral bacteria such as *Lactobacillus casei* and *Actinomyces naeslundii* in human carious dentin lesions. However, it is not known how the MDPB antibacterial primer could affect bacterial growth and metabolic function of cariogenic bacteria especially when they are in association [12]. Coaggregation of streptococci, *Actinomyces*, *Veillonella spp.* and *Fusobacterium nucleatum* were detected in oral biofilms [13] and it may reveal interaction of different bacterial species i.e. streptococci and *Actinomyces* [14] decrease the effect of antibacterial agents [12].

The purpose of this study was to investigate the effect of dentin bonding system containing MDPB after curing on selected oral bacterial viability and test its effect on single and multi-species bacterial suspension in vitro. The efficacy of the dentin bonding restorative material was assessed by bacterial viability using MTT and Live/Dead assays and also by measuring the metabolic activity of test bacteria and associations by the XTT assay. The hypothesis was that MDPB containing primer decrease the metabolic activity and viability of bacteria over the time tested in this study.

2. Material and methods

2.1. Bacteria

Streptococcus mutans 31377, *Streptococcus sobrinus* 27351, *Streptococcus gordonii* 10558, *Actinomyces viscosus* 19246 and *Lactobacillus lactis* 12314 were obtained from ATCC (Bethesda, MD). The bacteria were grown in Trypticase Soy broth (TSB, Difco, Detroit, MI, US) for 24 h at 37 °C. Bacteria were harvested by centrifugation and re-suspended in TSB and the number of bacteria per ml was determined by measuring the optical density at 600 nm and adjusted to a standard bacterial suspension of 1×10^7 CFU/mL.

2.2. Preparation of dentin bonding system discs

A low viscosity polyvinyl siloxane mold (Aquasil, Dentsply DeTrey, Konstanz, Germany) with a 6 mm diameter and 2 mm high was used to make dentin bonding system discs ($n = 3$). Discs of Clearfil SE Protect (CP) and Clearfil SE Bond (CB) were prepared according to the manufacturer's recommendation (Table 1). Briefly, one drop of self-etching primer was placed in the mold, and after 20 s one drop of bonding system was added to the primer and light cured for 20 s with Elipar Tri-light unit (3M ESPE – Seefeld 82229 – Germany). The polymerized discs were immersed in 0.5 mL of sterile distilled water immediately after preparation and agitated for 1 h to remove the uncured components. The methodology used in the present study was based on the study developed by Imazato et al., [5]. The disk specimens were placed into Eppendorf tubes with 150 μL of test bacterial suspensions, either single bacteria or mixtures of bacteria (1×10^6 CFU/mL) and incubated for 15, 30, and 60 min. At least three independent experiments were performed. At the end of each incubation period, the effect of the dentin bonding systems on bacteria was evaluated as described below.

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