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Antibacterial surface properties of fluoride-containing resin-based sealants

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ABSTRACT

Objectives: The aim of the present study was to determine the antibacterial properties of three resin-based pit and fissure sealant products: Clinpro (3M ESPE), Embrace (Pulpdent), and UltraSeal XT plus (Ultradent).

Methods: The antibacterial effects of the sealants were tested in both an agar diffusion assay and a planktonic growth inhibition assay using *Streptococcus mutans* and *Lactobacillus acidophilus*. The materials were applied to paper and enamel disks in the former and on the side walls of 96-well microtiter plates on the latter.

Results: All materials showed either diffusible or contact antibacterial effects in the agar diffusion assays. The effect was diminished when enamel disks were used as substrate. In the planktonic growth inhibition assay, Clinpro had its effect reduced, but retained activity against both bacteria over time. *L. acidophilus* was more sensitive than *S. mutans* to UltraSeal. Embrace retained antibacterial activity against both bacteria over time.

Conclusions: Within the limitations of this *in vitro* study it can be concluded that all materials are capable of contact inhibition of *L. acidophilus* and *S. mutans* growth. Embrace has the longer lasting antibacterial activity when in solution, especially against *S. mutans*.

1. Introduction

Sealants have been used for decades as a preventive measure against caries development in susceptible pits and fissures. Their beneficial effect, which has been recently corroborated in a meta-analysis,[1] depends largely on retention.[2] This mechanical retention is achieved by etching enamel with phosphoric acid,[3–5] which also reduces the bacterial count that may be present on the treated surfaces.[6] Once the area is sealed, the isolation of the bacteria underneath the sealant from oral nutrients helps minimize the development of caries.[7] It has been suggested that this positive effect could possibly be enhanced by adding fluoride to the sealant material.[8]

Fluoride is able to incorporate into enamel, making it more resistant to acid degradation; it also has antibacterial effects against cariogenic oral bacteria.[9] However, although it seems obvious that fluoride would improve the protective effect of sealants, equivocal results have been reported. Clinical studies where sealants were applied to caries-free surfaces have

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shown no significant difference in caries inhibition or retention rate between conventional and fluoride-containing sealants.[10–12] On the other hand, *in vitro* studies have demonstrated the potential inhibitory effect of some resin-based fluoride-containing sealants on cariogenic oral bacteria.[8,13,14] This potential antibacterial effect may be advantageous, for instance, when caries is inadvertently left in the surface to be sealed.[7]

In vitro studies testing antibacterial potential of materials are often performed with mutans streptococci and lactobacilli species, which are believed to be the two major groups of bacteria involved in the caries process.[9] In those groups are included *Streptococcus mutans* and *Lactobacillus acidophilus*, respectively. These bacteria produce acids, primarily lactic acid, that diffuse into the enamel and dissolve the mineral crystals to initiate the caries process.[15] This study does not evaluate the demineralization effects of those bacteria in enamel but uses enamel as a substrate in one of the experiments. To the best knowledge of the authors, no other study has used enamel as a substrate to study the antibacterial effects of sealants.

The purpose of this study is to evaluate the antibacterial surface properties of three commercially available resin-based fluoride-containing sealants using an agar diffusion test to determine the inhibition of surface growth and a planktonic growth inhibition test to determine the inhibition in broth culture. The hypothesis is that the resin-based fluoride-containing sealants have antibacterial activities under both test conditions against both *S. mutans* and *L. acidophilus*.

2. Materials and methods

Materials used in the study are listed in Table 1. They were tested against *S. mutans* strain ATCC 10449 (serotype c) and *L. acidophilus* strain ATCC 4356 using assays that measure the diffusible inhibition of bacterial growth on an agar surface and that estimate the antimicrobial activity by determining the inhibition of bacterial growth in broth culture. While the first experiment studied the ability of the materials to inhibit bacterial growth on a surface (simulating plaque formation), the second determined the materials' action against the bacteria in liquid suspension (such as saliva). All procedures were performed under aseptic conditions. The same operator applied the materials in all of the assays.

2.1. *Agar diffusion assay—paper disk*

Each material was applied to 6-mm sterile paper disks (Becton Dickinson and Company, Sparks, MD, USA) using the sealants' applicator. Approximately 12 mg of the materials were placed onto the paper disk and light-activated for 20 s using a Demetron A.2 LED curing device (Kerr Corporation, Orange, CA, USA) with an output of 1000 W/cm^2. Blank disks (no material applied) were used as controls.

Inocula from frozen stock cultures were cultivated in Wilkins-Chalgren (W-C) broth (Oxoid Ltd., Basingstoke, Hampshire, England, UK) at 37 °C in ambient atmosphere, after being screened by Gram-staining to confirm purity. Loopful inoculations of *S. mutans* and *L. acidophilus* were transferred to 9 mL of appropriate broth and incubated at 37 °C under anaerobic conditions. Bacterial suspensions were prepared to 0.5 MacFarland standard[16] and diluted to a 1:10 concentration with W-C broth. Two hundred microliters of the 1:10 dilution were then taken and spread-plated using a ''hockey stick'' on a turntable to ensure confluent bacterial distribution on the plates.

Test specimens were immediately placed on the freshly inoculated agar plates and aerobically incubated for 48 h at 37 °C. Each plate contained four disks; one of each sealant group and a blank disk. This assay was performed four times. The diameters of the zone of inhibition of bacterial growth around the disks were measured using a caliper. If a zone of inhibition was not evident, the disk was removed to determine if there was growth under it. If inhibition was evident under the disk this outcome was treated as a qualitative observation indicating retention of antibacterial activity on the contacted surface. Diffusible activity was defined as a zone of inhibition greater than the 6-mm diameter of the disk. Both bacterial strains tested yielded confluent growth under the blank disks.

2.2. *Agar diffusion assay—enamel disk*

Thirty-two enamel disks (6 mm in diameter and 2 mm thick) were cut from the labial surface of 32 lower anterior bovine teeth and were sterilized using ethylene oxide gas at the beginning of the experiment. The enamel surfaces of the disks were acid-etched with 35% phosphoric acid (Scotchbond Phosphoric Etchant, 3M ESPE, St. Paul, MN, USA) for 15 s, thoroughly rinsed with distilled water, and dried with compressed air. Materials were applied and the tests

Table 1 – Materials composition.

Material	Type	Manufacturer	Composition
Clinpro sealant	Pit and fissure sealant	3M ESPE, St. Paul, MN, USA	Triethylene glycol dimethacrylate, bisphenol A diglycidyl ether dimethacrylate, tetrabutylammonium tetrafluoroborate, silane treated silica
Embrace WetBond pit and fissure sealant	Pit and fissure sealant	Pulpdent Corporation, Watertown, MA, USA	Di–tri multifunctional monomers in an acid-integrating network, fluoride
UltraSeal XT plus	Pit and fissure sealant	Ultradent Products, Inc., South Jordan, UT, USA	Diurethane dimethacrylate, bisphenol A diglycidyl ether dimethacrylate, fluoride