Microleakage and Antimicrobial Properties of Experimental Antibacterial Fluoride-Releasing Dental Sealants

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ABSTRACT

Purpose: The purpose of this study was to investigate the antimicrobial properties and microleakage of experimental antibacterial fluoride-releasing sealants. Methods: Two commercial non-antibacterial sealants Clinpro™ (3M ESPE) and Ultradent XT Plus™ (Ultradent) were used as Control-1 and Control-2. Two experimental sealants (Exp-1, Exp-2) were prepared by adding 10% of a synthesized antibacterial fluoride-releasing monomer C16DC2DMA to the two controls, respectively. A microleakage test for all four groups was conducted on extracted teeth using a dye-penetration method. A *S. mutans* biofilm assay was conducted on all groups using a direct contact assay and drop-plating. Two-factor linear mixed models with random effects for tooth IDs were used to analyze differences in microleakage scores between treatment groups while accounting for repeated measures on each tooth. Pairwise comparisons were made between each treatment group using this mixed model and Wald tests. Results: None of the microleakage scores were significantly different between pairs of treatment groups(p>= .071) but the two experimental sealants showed significant reduction (by 3 to 7 orders) in biofilm CFU when compared with controls. Conclusion: The synthesized antibacterial fluoride releasing monomer C16DC2DMA can be used to generate antibacterial sealants with significant biofilm inhibition effect.

INTRODUCTION

Caries in young children remain the most common chronic disease.[1]The two forms of prevention include primary prevention and secondary prevention. Primary prevention involves interventions that attempt to avoid the onset of caries. Secondary prevention involves interventions that try to avert the progression of early caries to cavitation. The pits and fissures on occlusal surfaces of teeth are generally too narrow for a manual toothbrush to access therefore making them more prone to develop dental caries.[2] Hence, sealing the occlusal surfaces with trustworthy dental sealant materials is an American Academy of Pediatric Dentistry (AAPD) accepted preventative measure to help prevent dental caries.[3] Sealants are placed to help prevent caries initiation and to arrest caries progression by forming a physical barrier that inhibits microorganisms and food particles from congregating in the pits and fissures of teeth.[3]Microleakage occurs when there is a poor bond to tooth structure due to faulty isolation, recurrent decay, or user error. It is known that the less microleakage present, the better the seal is to tooth structure therefore increasing the quality and longevity of the dental sealant.[4] This is why microleakage was quantified to ensure that the experimental dental sealants are of good quality. By introducing antibacterial monomers into dental materials, we can try to combat recurrent decay and prolong the life of dental restorations.[5]

METHODS

Microleakage test

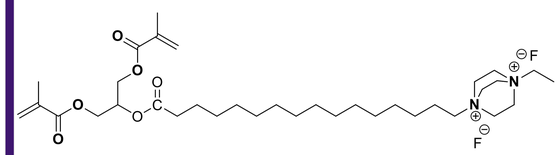
The microleakage test was conducted by one researcher to reduce variability. There was a total of 4 groups of extracted teeth that consisted of 7 mandibular molars stored in 10% Sodium Thymol solution. A 1.00 mm fissure was placed into each central groove of the teeth by using a 330 burr and high-speed hand piece. Each tooth was then etched using Ultra-Etch 35%™ phosphoric acid gel solution for 30 seconds. The teeth were then rinsed with water and dried. Sealant material of each group was applied and cured for 40 seconds(s) and checked with an explorer. The unsealed side was coated with nail polish and thermocycled for 2,000 cycles at 5˚C to 55˚C. Specimens were then stained using 2% basic fuchsine for 24 hours, rinsed, air-dried, and embedded in epoxy resin. Specimens were sectioned mesiodistally into 6, 2 mm thick slabs, observed, and photographed under a microscope. Microleakage scores of 0 to 4 were assigned by the primary investigator(0=no penetration; 1=1/4 penetration; 2=1/2 penetration; 3=3/4 penetration; 4=penetration into the bottom of the fissure) as shown in Figure 2.

Direct Contact Biofilm Assay

A modified direct contact biofilm assay was conducted to measure the effectiveness of the experimental antibacterial monomer against *Streptococcus mutans.* The drop plating method was used to examine the viable cell count. Round sample disks were made of each group. *S. mutans* was inoculated on each disk and allowed to dry. The samples were then transferred to 3 mL Brain Heart Infusion (BHI) agar for 22-24 hours of incubation. The supernatant was removed from each tube the following day. The samples were triple washed then transferred to sterile BHI agar. Sonication was used to detach cells from the sample surfaces. Dilution of the detached cells was then performed with BHI agar. The detached cells were then drop plated onto BHI agar then incubated for 22-24 hours. The colonies were finally counted the following day.

RESULTS

Figure 1. Structure of the Synthesized Antibacterial Monomer



1-(16-((1,3-bis(methacryloyloxy)propan-2-yl)oxy)-16-oxohexadecyl)-4-ethyl-1,4-diazabicyclo[2.2.2]octane-1,4-diium fluoride

C16DC2DMA

Figure 1. Structure of the synthesized antibacterial monomer C16DC2DMA [26,27].

Figure 2: Microleakage Scoring

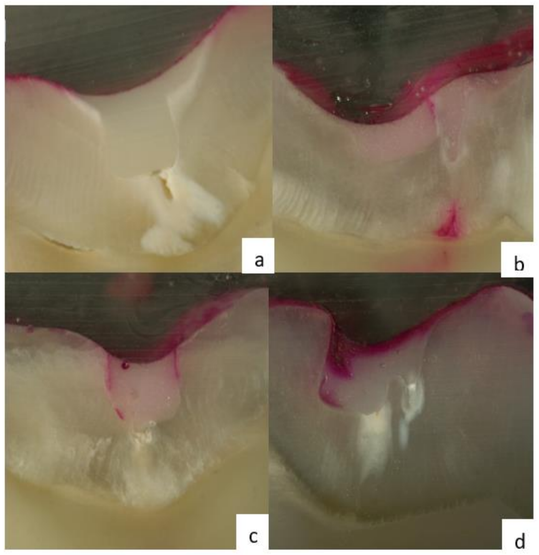


Figure 2. Example micrographs of sealants and their microleakage scores. (a) score=0, (b) score=1, (c) score=2, and (d) score=4. All images are under 40× magnification.

Figure 3. Associations between estimated scores and microleakage scores.

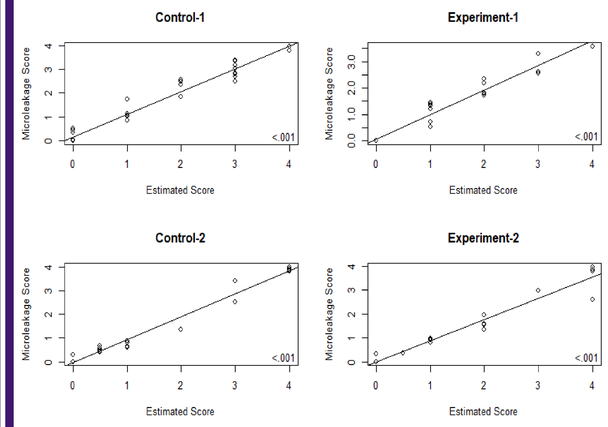


Figure 3. The estimated associations of estimated scored when compared to the calculated scores were 0.96 for Exp-1 group, 0.90 for the Control-1 group, and 0.95 for both Exp-2 and Control-2 groups.

Figure 4: Direct-Contact Biofilm Assay

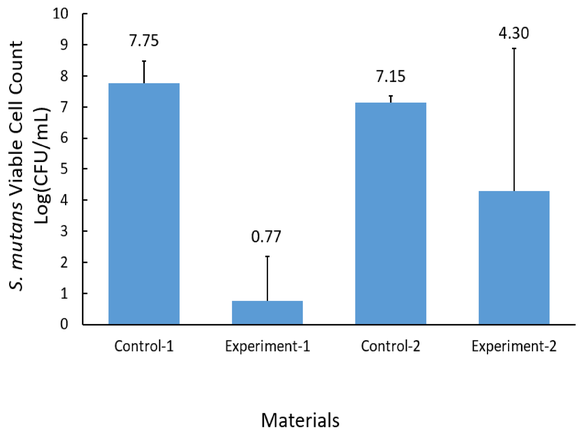


Figure 4. S. mutans biofilm viable count in log(CFU/mL) from the modified direct contact assay performed. The Exp-1 group was significantly different from the Control-1 group ($p<0.05$).

Table 1. Microleakage Scores

Materials	Cumulative Microleakage Scores
Control-1	1.56
Control-2	1.01
Exp-1	0.93
Exp-2	0.80

Table 1. Microleakage scores for the different sealants. Top to bottom the average scores were 1.56 (SD:1.44, 95% CI = .89, 2.35), 1.01 (SD:1.11, 95% CI = .20, 1.63), 0.93. (SD:1.49, 95% CI = .91, 1.16), 0.80 (SD:1.22, 95% CI = -.07, 1.40).

CONCLUSIONS

1. The new experimental antibacterial monomer, C16DC2DMA, did not compromise the physical properties of the sealants while having the extra protection of antibacterial properties.
2. The experimental sealants showed very little to no microleakage. Therefore, they are expected to have good retention.
3. The antimicrobial and biofilm-inhibitory properties of the experimental sealants were stronger than the commercial sealants.

ACKNOWLEDGEMENTS

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