Biofilm-inhibition and Microleakage Properties of Experimental Antibacterial Sealants

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INTRODUCTION

Dental caries is the most common chronic disease of childhood and is a multifactorial disease that requires a suspectable host, an active immune response, dietary sugar intake, and sufficient time. Due to the primary role that bacteria plays in the caries formation process, an antimicrobial approach shows the most promise for prevention. Other studies have been conducted in an attempt to increase the anti-bacterial activity of sealants and adhesives. The addition of fluoride, chlorohexidine, silver nanoparticles, and bioactive glass has shown promise. The purpose of this study was to explore the antibacterial properties and microleakage of three experimental antibacterial sealants compared to Clinpro 3M.

OBJECTIVES

Investigate the biofilm-inhibitory effects and microleakage properties of the experimental sealants containing Ga-doped bioactive glass (Ga-BAG) and an antimicrobial monomer compared to the control, Clinpro 3M.

METHODS

- ***Sample Preparation:***
  Three experimental antibacterial sealants were formulated by adding different antibacterial components to 3M ESPE ClinproTM sealant. 3M ESPE ClinproTM sealant served as the control. Group 1 was made of 20 wt% Ga-BAG , group 2 was 2 wt% synthesized antibacterial monomer (AMII), and group 3 was 20 wt% Ga-BAG and 2 wt% AMII.
- **Biofilm Assay:**
  The antibacterial activity of light-cured sealant discs (10 mm diameter, 2 mm thick, n=3) against S. mutans biofilm was tested using a modified direct-contact biofilm assay (Imazato, 2009) and Wang et al. (2018). The drop-plating method was used to examine the viable cell counts. Round sample disks were made of each group and S. mutans were inoculated on each disk and allowed to dry. The samples were then transferred to 3 mL BHI 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. Sonication was used to detach cells from the sample surfaces. Dilution of the detached cells was then performed with BHI. The detached cells were then drop plated onto BHI agar then incubated for 22-24 hours. The colonies were counted the following day.

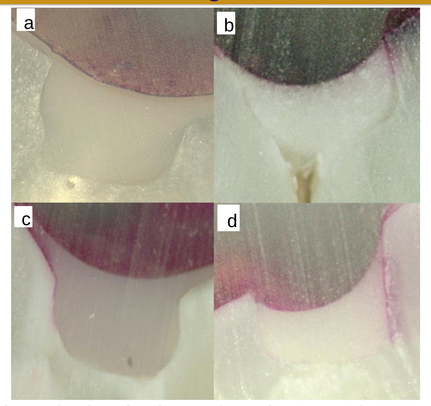
Microleakage Test:

- Twelve third molars, without occlusal caries, were randomly separated into four groups of 3. The teeth were stored in a 10% Sodium Thymol solution. A 1.00 mm fissure was placed into the central groove of each tooth. Each tooth then underwent sealant placement following product recommendations.
- Each tooth was etched using Ultra-Etch 35% phosphoric acid gel solution for 30 seconds. The teeth were rinsed with water and air-dried. Opti-bond was scrubbed on each tooth for 20 seconds, air-dried and light cured. Sealant material was applied and cured for 40s and checked with explorer.
- The unsealed side of the tooth was coated with nail polish and subjected to thermocycling for 2,000 cycles at between 5˚C and 55˚C. Specimens were stained using 2% basic fuchsine for 24 hours, rinsed, air dried, and embedded in epoxy-resin.
- Specimens were sectioned mesio-distally into 5, 1 mm thick slabs, observed, and photographed under a microscope. The estimated microleakage scores of 0 to 4 were assigned: 0=no penetration; 1=1/4 penetration; 2=1/2 penetration; 3=3/4 penetration; 4=penetration into the bottom of the fissure.
- The total depth of the sealant and the depth of dye penetration was measured and the calculated scores = 4x penetration depth/(total depth) was compared with the estimated scores.

Data Analysis:

The One-Way ANOVA test was performed to assess for statistically significant difference. P-value was set at <0.05. The Tukey's HSD was used to make stepwise comparisons between groups.

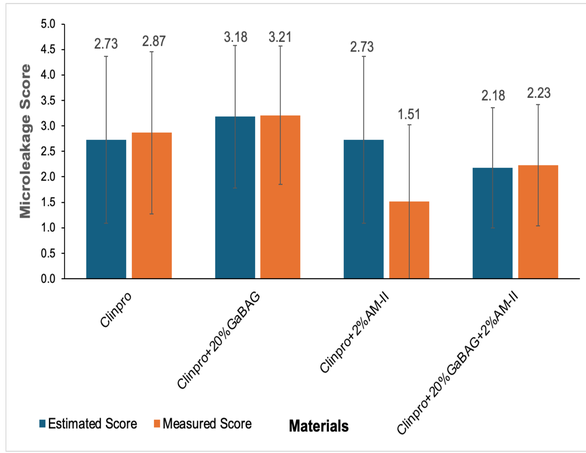
Figure 1. Microleakage Penetration



Examples of microleakge of sealants under microscope with 40x magnification and their related score. (a)=score of 0, (b)=score of 2, (c)=score of 3, (d)=score of 4.

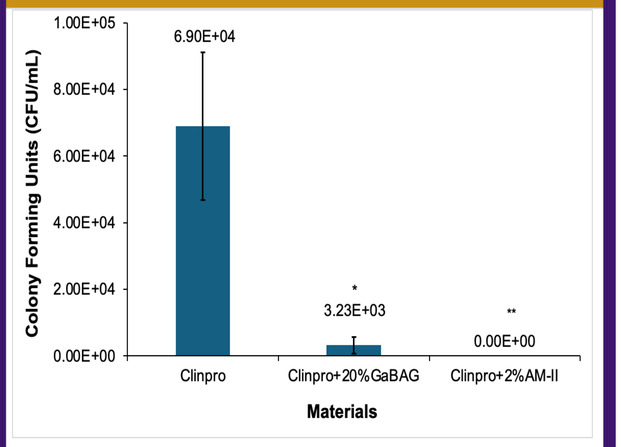
RESULTS

Figure 2. Microleakage Scores



The One-way ANOVA showed there was a significant difference between the experimental groups (p value<0.001).

Figure 3. Results of Biofilm Assay



Tukey's HSD was applied to preform stepwise comparisons between each group.

CONCLUSIONS

Summary:

• For microleakage test, the Clinpro+2 wt% AMII group had the lowest mean/median scores compared to the others.
•The microleakage in Clinpro+20 wt% Ga-BAG+2 wt% AMII group had a lower score than Clinpro and Clinpro+20 wt% Ga-BAG but the difference is not significant.
• For biofilm assay, the experimental sealants containing 20 wt% Ga-BAG or 2 wt% AMII had significantly lower S. mutans biofilm CFU than the control (Clinpro) indicating their significant biofilm-inhibition effects.

Conclusion: The sealant containing 2 wt% AMII showed the strongest biofilm-inhibition effect and the lowest microleakage. A sealant of this nature may have better caries prevention.

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