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Tooth Micro-hardness Changes After Applying Bioactive Glass-containing, Anti-microbial Sealants

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ABSTRACT

The AAPD recommends placement of dental pit and fissure sealants on surfaces that are high risk or that already exhibit incipient carious lesions. Protection provided by these sealants may be enhanced by the addition of ion-releasing, anti-microbial filler particles of bioactive glass.

Objective: We prepared novel dental pit and fissure sealant materials containing bioactive glass (BAG) fillers and tested their ability to prevent tooth demineralization in a bacterial broth.

Methods: Two types of BAG were synthesized in our lab:
BAG1 (61 wt% silica - 31 wt% calcia - 4 wt% phosphate - 4 wt% flouride);
and BAG2 (81 wt% silica - 11 wt% calcia - 4 wt% phosphate - 4 wt% flouride).

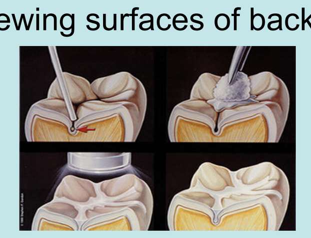
Ultraseal XT (USXT) resin without filler was supplied by the manufacturer (Ultradent Products, Inc. South Jordan, UT). BAGs were individually incorporated into the resin (25 wt%) and provided handling properties similar to USXT. Caries-free teeth (n=5 each) were randomly assigned to three groups (BAG1-sealant, BAG2-sealant,or USXT) and sealants were placed by the same practitioner. Acid-resistant nail polish was used to cover half of the tooth surface. Teeth were immersed in a bacterial culture system of sucrose-rich brain-heart infusion (BHI) media containing Streptococcus mutans strain #25175, an acid-producing microbe and incubated at 37˚C, 5%CO_2; media was changed every other day. Bacterial growth was confirmed throughout the test period. At two weeks, teeth were sectioned sagitally and microhardness testing compared changes in hardness as a function of location on the tooth.

Results : Overall, the BAG2-sealant samples were significantly harder than the BAG1-sealant or USXT samples. Areas adjacent to the BAG2-sealant were harder than the original tooth surface. (ANOVA/Tukey's; α=0.05).

Conclusion: The inclusion of anti-bacterial, ion-releasing BAG as a filler component results in a harder tooth that may be better able to resist demineralization.

INTRODUCTION

Oral diseases are the most common preventable disease in United States today, while dental caries is the most common chronic disease of childhood. Sealants are one way of preventing bacteria from getting to the teeth and are effective in preventing pit and fissure caries. Children who receive sealants are 72% less likely to receive restorative services over the next 3 years then children who do not. These sealants protect the grooved surfaces of the teeth, and the chewing surfaces of back teeth where most cavities are found. Research shows that sealants can last for up to 3 years if properly cared for but can wear off and leave teeth vulnerable. Research has confirmed that within 26 months, most sealants fail clinically.



This project developed a new type of sealant that releases fluoride, calcium and phosphate ions. These ions strengthen the tooth and kill bacteria. This new type of sealant was prepared by combining Sol-Gel Bioactive Glass [BAG] with an adhesive monomer. This new BAG sealant will not only protect the tooth for an extended period of time, but also strengthen the tooth, and kill bacteria before they can attach to the tooth surface. BAG is a three-dimensional network of silica, calcia, phosphate, and fluoride ions, so in addition to working as an antibacterial agent, it also strengthens teeth and enhances regeneration of tooth structures. In this study we used two different BAG formulations: BAG81 which has a strong structure and BAG 62 for its higher concentration of calcium and phosphorus. Both compositions contain releasable fluoride.

Sealants that are currently in use don't have this beneficial ion-release, particularly the anti-microbial benefit. Some studies have demonstrated that S. mutans will accumulate and propagate more readily on surfaces of cured composite resin restorations than on the surfaces of restorations made with other materials. Therefore, restorative materials with antibacterial properties may play an important role in controlling the activity of such bacteria. Additionally, as sealants age in use, they develop cracks that form perfect sites for bacteria to attach and grow. These new sealants should help prevent colonization from occurring in these sites.

MATERIALS & METHODS

Tooth Preparation

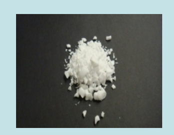
A total of 40 unerupted molars with no evidence of existing caries were selected. All teeth had been removed for clinical purposes and were stored in 0.5% Chloramine-T for less than 6 months prior to use. All teeth were scaled to removed adherent soft tissues and were mounted in dental stone for sectioned to remove the intact crown using an Accutom-5 (Struers Corp., Westlake, OH) low speed rotary saw with a diamond wafering blade.

Residual pulp tissue was removed from the crown and the pulp chamber and all exposed dentin surfaces were covered with flowable composite (Filtek Supreme Ultra, 3M-ESPE, MN) and packable composite (Z100, 3M-ESPE, MN). The apical portion of the sample was coated with acid-resistant varnish (Revlon Nail Enamel, Revlon Corp, New York) up to the cemento-enamel junction.

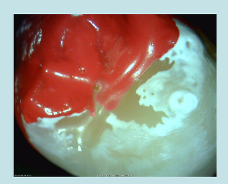
Preparation of sealant

Two types of bioactive glass (BAG) were prepared in our laboratory using a sol-gel process, aged, ground and micronized to a particle size less than 3.0 um. BAGs had compositions as follows:BAG81 = 81% SiO_2/11% CaO/4% P_2O_5/4% F;
BAG62 = 61% SiO_2/31% CaO/4% P_2O_5/4% F.

A Siemens Speed Mixer (DAC 150 FVZ, FLakTek Inc, SC) was used to blend Ultraseal XT plus monomer without filler (obtained from Ultradent Products, UT) with the BAGs in approximately a 30% filler load. This provided a handling consistency similar to conventional Ultraseal XT, which was used as a control material.



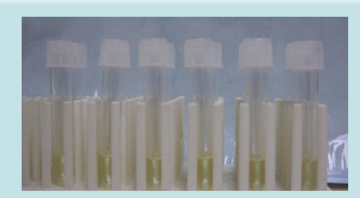
Sealant application



An experienced pediatric clinician performed all sealant application procedures. In brief, teeth were etched with phosphoric acid gel for 20 seconds, rinsed and the sealant was applied using a microbrush. The sealant was cured for 20 seconds using an LED curing light (Demi, SDS Kerr, WI). Following curing, each sample had the sealant surface brushed vigorously using a slurry of 48 g. of Crest ProHealth for Me toothpaste mixed with 100 mL of water. Samples were rinsed with deionized water and blotted dry. Acid-resistant varnish was placed over one half of the coronal surface, covering approximately half of the sealant.

Bacteria

Streptococcus mutans was obtained from ATCC (strain 25175). The bacteria was grown in brain-heart infusion (BHI) media, to which 3% sucrose was added. Colonies were triple-cultured to ensure only a single species was present and used in logarithmic phase. One half of the prepared samples were individually immersed in 3 ml of bacterial media and stored in an incubator at 37ºC, 5% CO_2. Media was changed every other day.



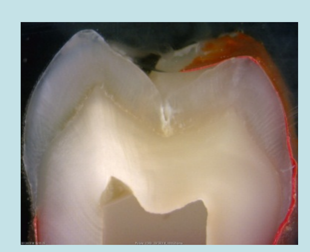
pH cycling solutions

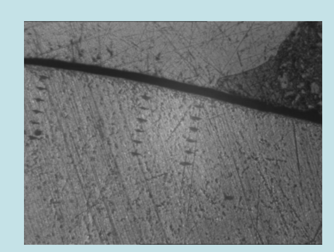
Demineralizing and remineralizing solutions were prepared following a procedure originally described by Featherstone, et al. The remineralization solution contained $CaCl_2$, K_2HPO_4, KCl, and Na Cacodylate in deionized water, adjusted to a pH of 7.0. The demineralization contained acetate buffer, NaO_2CCH_3 and $CaHPO_4$ in deionized water, adjusted to a pH of 4.5. Twenty of the prepared samples were used for pH cycling and the remaining twenty were subjected to bacterial challenge. For pH cycling, the samples were were immersed in 3 mL of the remineralization solution for 18 hours, followed by a brief rinse and immersion into the demineralization solution for 6 hours. This sequence was repeated for a total time of 15 days at 37ºC.

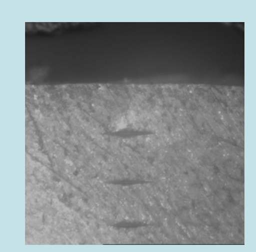
Analyzing the samples

Following the challenge presented to the samples (both acidic and bacterial), the teeth were rinsed and blotted dry. They were embedded in acrylic resin and sectioned perpendicularly through the coronal surface using the low speed rotary saw. The cut was placed so as to allow visualization of the cross-sectional area beneath the crown of the following regions:

1: under the varnish, but not under the sealant – this was considered our control region;
2: under the varnish, at the edge of the sealant;
3: under the sealant, but not under the varnish;
4: at the edge of the sealant, exposed to the cycling solutions;
5: Away from the sealant, exposed to the cycling solutions.







Microhardness indentations were made under each of these regions using a knoop indenter (Duramin-5, Struers Corp, OH). Indentations were spaced 50 um apart, going perpendicular to the tooth surface. A total of 10 indentations were made at each location on each sample.

RESULTS

- Overall, the BAG81-sealant samples were significantly harder than the BAG61-sealant or USXT samples.
- Areas adjacent to the BAG81-sealant were harder than the original tooth surface.

- Results from the two challenge methods were similar, however the following slight differences were observed:
  - In the bacteria samples, areas 2 an 3 were significantly harder than areas 4 and 5.
  - In the pH cycling samples, area 2 was significantly harder than areas 4 and 5

ANOVA with repeated measurement in SAS with pair-wise post hoc comparisons was performed between 3 materials, between areas and between depth for chemical and bacteria treatments, respectively.

DISCUSSION

Release of fluoride has benefits as it has antibacterial properties and increases the acid resistance of enamel due to the formation of fluoroapatite. The chemical composition of BAG-containing sealants allows the release of high concentrations of calcium, phosphate, and fluoride potentially inhibiting enamel demineralization.

USXT does not release ions that inhibit demineralization. As expected, samples sealed with USXT had the most demineralization and visually showed the most apparent white spot lesions, with both bacterial and chemical challenge. BAG-61 sealant showed significantly less demineralization than USXT at ??? indentations. Samples bonded with fluoride and calcium-releasing BAG62 sealant had the least demineralization and visually showed the least apparent white spot lesions. In fact, enamel below the BAG-containing sealants demonstrated an increase in microhardness (or mineralization) relative to control enamel.

Ideal sealant materials should have sustained ion release to aid in the protection against demineralization while demonstrating sufficient bond strength to protect the underlying tooth surface. These new bioactive sealants may be most advantageous in patients with poor oral hygiene, as they may show unpredictable compliance with topical fluoride or fluoride mouth rinses and therefore would benefit from the intrinsic ion release.

CONCLUSIONS

- BAG-containing sealants were associated with significantly lower superficial demineralization than USXT.
- BAG61 sealant samples where harder than the BAG81 sealant or the Ultraseal-XT samples, indicating that the BAG61 composition may likely have an advantage over the other two sealants.
- The inclusion of ion-releasing BAG as well as it's anti-bacterial component gives a result of harder teeth that can resist demineralization better.
- Patients with poor oral hygiene may benefit from sealants that release calcium, phosphate, and fluoride

ACKNOWLEDGEMENTS

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