Acrylated Hydroxyazobenzene Coating Over Resin-based Sealants Inhibits *Streptococcus Mutans*

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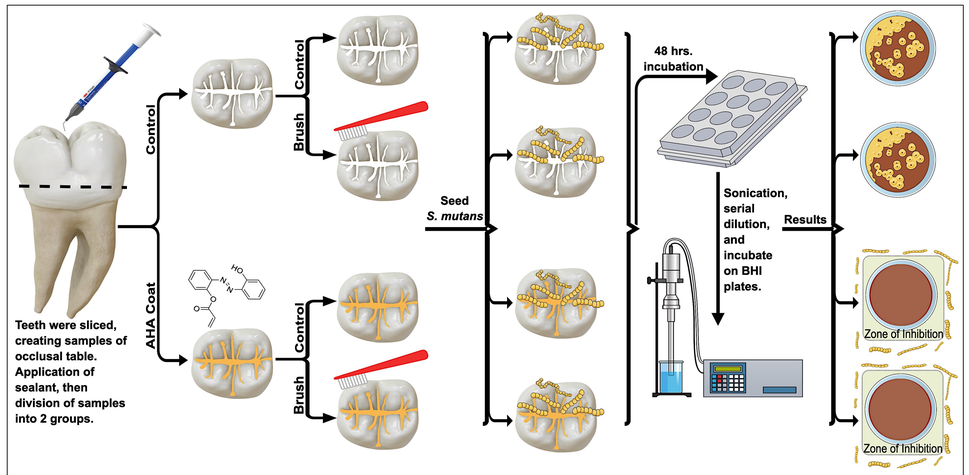
Background

- Dental caries is a preventable chronic infectious diseases leading to pain, infection, loss of function, and malnourishment.[1,2,3]
- Dental caries is a dynamic disease process involving bacteria, such as *Streptococcus mutans (Sm),* refined carbohydrates, tooth substrates, and time.[1,4,5,6]
- Sealants create a physical barrier that prevents biofilm growth by blocking nutrients, and their use on first permanent molars prevents caries.[7,8]
- Sealants wear and detach overtime leading to biofilm formation and subsequently caries around the sealants.[4,5]
- Azobenzene-derivatives have demonstrated antibacterial properties in many fields, including healthcare.[10,11]
- We have developed and used a novel acrylated hydroxyazobenzene (AHA) molecule that has demonstrated antibacterial properties against *Sm.*[12,13,14]
- This study hypothesized that an AHA coating over dental sealants would demonstrate inhibition of *Sm*, *in vitro*.
- The primary objective of the study was to evaluate the efficacy of dental sealants with AHA in inhibiting *Sm* growth.
- The secondary objective was to study the retention of the AHA-mediated inhibition of *Sm* growth after simulated toothbrushing.

Methods

- Commercially available resin-based sealant (3M™) was applied on the occlusal surfaces of extracted human molars (n=12) and cured for 40 seconds. One group of samples received AHA coating (4.2±0.4µL within solvent) over sealants followed by 40 seconds curing.
- AHA-coated (test) and uncoated molars (control) were further subdivided into two groups with or without toothbrushing (equivalent to six-months).
- All the samples were subsequently washed with 70% ethyl alcohol for 15 minutes, followed by 5 minutes of UV irradiation.
- Substrates (sealed molars ± AHA coating) were incubated in phosphate-buffered saline containing 1% Penicillin-Streptomycin overnight and then washed.
- *Sm* (10^7) were seeded on the substrates and cultured for 24hrs at $37°C$ and 5% CO in Brain Heart Infusion Agar (BHI) with 1% sucrose. The media was replenished after 24 hours.
- At 48 hours, substrates and surrounding media were sonicated, followed by a serial dilution which was seeded onto BHI plates.
- *Sm* on the substrates and surrounding media were quantified by counting colonies of *Sm*.



Results

- The AHA-coated molars and the surrounding media did not demonstrate growth of a single *Sm* colony on BHI plates while control substrates demonstrated uninhibited growth of *Sm*.
- Similarly, compared to the controls, the dilution from AHA-coated molars after six months of toothbrushing and the surrounding media did not exhibit growth of a single *Sm* colony.
- The dilutions from the uncoated molars and the surrounding media, regardless of toothbrushing, demonstrated the proliferation of abundant *Sm* colonies ($\sim 3 \times 10^5$ for brushed) on BHI plates.
- The AHA coating was intact even after six months of simulated tooth brushing.

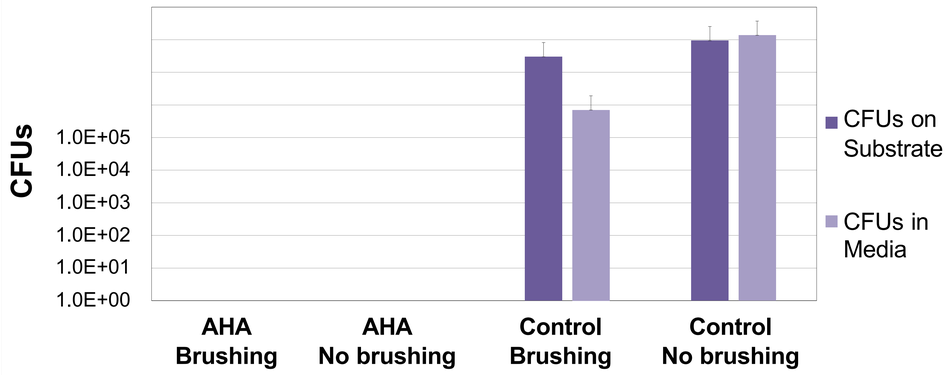


Figure 1: Colony forming units of *Streptococcus mutans* from substrates with sealants (control) with AHA coating (test) with or without six months of simulated tooth brushing.

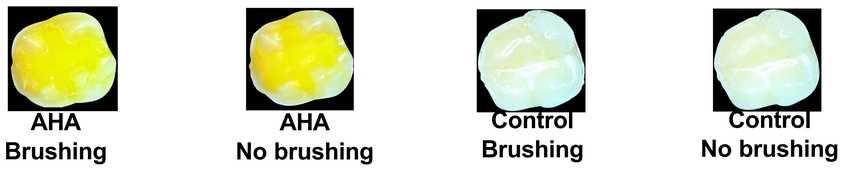


Figure 2: Photographs of substrates with dental sealants (control) or with AHA coating (test) with or without six months of simulated tooth brushing.

Discussion

- Untreated caries are the cause of 12% of global productivity losses.[6,15,16]
- Due to the complex occlusal morphology, toothbrushing does not effectively remove plaque from the pit and fissures and hence, 90% of the carious lesions initiate in the pits and fissures.[2]
- The application of sealants is an effective method to prevent dental caries developing on the occlusal surface.[9,17,18]
- Partial or complete loss of sealants overtime leads to biofilm formation and subsequently caries around the sealant.[3,6,19]
- Commercial fluoride-releasing sealants may provide antibacterial properties. However, the frequency of carious lesions around fluoride sealants is comparable to the non-fluoridated sealants.[6,19,20]
- Incorporation or coating of antibacterial compound over sealants would prevent caries around partially or completely lost sealants. However, the major drawback of this modality is that such an antibacterial effect is only dose-dependent.[6,12,13,14,20]
- This is the first study to evaluate the effect of AHA coating over dental sealants, along with the retention of antibacterial inhibition over a longer observation period.
- AHA in minimal concentration promotes *Sm* inhibition over an extended period. It is advantageous over other antibacterial additives, which require larger doses, resulting in compromised bond strength at the tooth-sealant interface.
- The *in vitro* nature of study can be considered as a limitation and needs further *in vivo* confirmation of this effect.
- While small sample size can be considered as a limitation, the controlled nature of study, use of biologic replicates, and observed difference of *Sm* inhibition strengthen the significance of the study findings.

Conclusions

- The efficacy of AHA coating in complete inhibition of *Sm* was evident with or without six-month equivalent of toothbrushing.
- The retention of AHA coating after a six-month equivalent of toothbrushing demonstrated no impact on bonding of sealant.
- The AHA coating exhibited inhibition of *Sm* in the surrounding Media confirming a "zone of inhibition" around the AHA coating.
- The efficacy of AHA coating needs to be confirmed *in vivo*.

References

Complete list of references available upon request.

Acknowledgements

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