Inhibition of *Streptococcus mutans* through Hydroxylated Azobenzene Compounds as applied to 3D Printed Dentures

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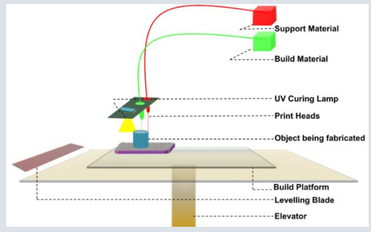
ABSTRACT

Streptococcus mutans is a gram-positive coccus bacteria found in the oral cavity and contributes significantly to tooth decay. Dentures can harbor *S. mutans* along with other bacteria, causing high rates of oral cavity infections among their wearers.

Despite advances in 3D printing and dentistry, the direct fabrication of robust long-lived components of full/partial dentures remains an unmet goal. The shift in fabrication methods to 3D printing opens the door to lower costs and more rapid production of removable partial dentures (RPDs) for these patients.

The objective of this work is to formulate a polymer for a 3D printed removable partial denture base with appropriate flexural modulus and strength, containing an acrylated hydroxyazobenzene (AHA) compound for the inhibition of carries causing *Streptococcus mutans*. We have demonstrated that at 0.25% wt. concentrations in resin, AHA has an inhibitory zone effect on *S. mutans*. This inhibition is also seen in media, where complete inhibition of 10^5 cfu/mL starting culture was demonstrated at 5 hours in 500 μL BHI. The 46% urethane dimethacrylate (UDMA), 37% ethylhexyl acrylate (EHA), and 17% methacrylic acid (MAA) cured polymer, with and without the 0.25% wt. addition of AHA, has been confirmed to be non-toxic to mouse fibroblast cells and has demonstrated ideal flexural strength and modulus properties for application in the construction of 3D-printed removable partial dentures.

INTRODUCTION AND PURPOSE

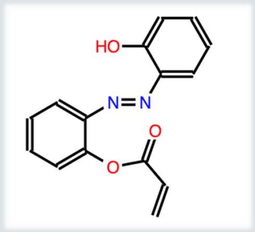


(Guddati et al., 2019)

(Valplast)

Streptococcus mutans is a gram-positive coccus bacteria found in the oral cavity and contributes significantly to tooth decay. The Nair lab has demonstrated that acrylated hydroxyazobenzene (AHA) in resin polymer has an inhibitory effect on *S. mutans* at 2 wt% (Mori et al., 2020). The Stansbury lab has developed a new polymer with ideal mechanical and physical properties for use in the 3D inkjet printing of removable partial dentures. The goal of this project is to create a UV-curable polymer with the lowest possible concentration of AHA while maintaining an inhibitory effect on S. mutans for application in the 3D printing of dentures.

MATERIALS & METHODS



Acrylated HydroxyAzobenzene (AHA)
MW = 258.27 g/mol

Resin Formulations:

The RPD formulation was created using 46% urethane dimethacrylate (UDMA), 37% ethylhexyl acrylate (EHA), and 17% methacrylic acid (MAA) by weight. To the RPD resin, different concentrations of AHA including 0.0 wt%, 0.25 wt.%, 0.5 wt%, 0.75 wt%, and 1 wt% were added.

Sample Curing:

The samples were polymerized using a redox reaction (0.5% NNDpT/1% BPO) followed by a thermal post-cure for 1 h at 80°C (using 1% AIBN thermal initiator).

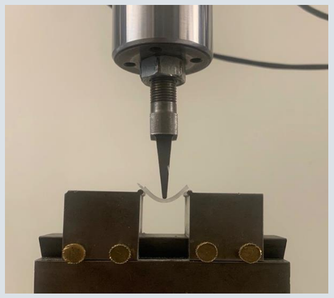
S. *mutans* Kill Curves:

Streptococcus mutans liquid cultures were diluted to 10^5 CFUs/mL. 0.25 wt% AHA in resin and control pucks were sterilized and placed into 500 μL of BHI media. 20 μL of media was sampled every hour and live bacterial concentrations were quantified using serial dilutions and seeding on agar plates.

Cytotoxicity:

Direct and indirect AHA and control samples were assessed using MTT Assays with L929 Mouse Fibroblasts.

A 2x2 mm bar of polymerized resin undergoing a 3-point bend test on the Materials Testing System machine



Mechanical Strength Tesing:

2x2 mm resin bar samples were subjected to 3-point bend testing on the Materials Testing System (MTS) in both, dry and wet (DI H_2O at 37°C bath for 48 hours) conditions to measure flexural strength and modulus.

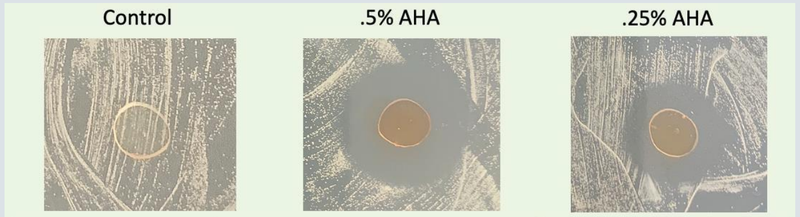
Conversion Measurements:

Conversion of cured samples was measured using near-infrared spectroscopy by taking a ratio of the reacting (meth)acrylate peak for each sample to the unreacting urethane peak. This ratio was then compared to that of an uncured sample to determine degree of conversion in the polymer.

Inhibitory Zone Determination:

Inoculations of *S. mutans* in BHI were grown in the incubator at 37°C for 16 hours. The culture was then diluted 1:10 in BHI and 100 μL of media was spread on an agar plate. 3 resin pucks were placed on the plate and incubated for 24 hours. The zone of inhibition was determined using AM scope software.

RESULTS



Sample	Average Inhibitory Zone N=3, n=3	Post-Cure Conversion
Control	0.00 ± 0.00 mm	98.0%
.25% AHA pucks	12.5 ± 0.96 mm	96.9%
.5% AHA pucks	15.4 ± 2.4 mm	92.9%

Figure 1. The 0.25 wt% AHA formulation successfully demonstrated a *S. mutans* inhibitory zone and thus was selected for successive investigations.

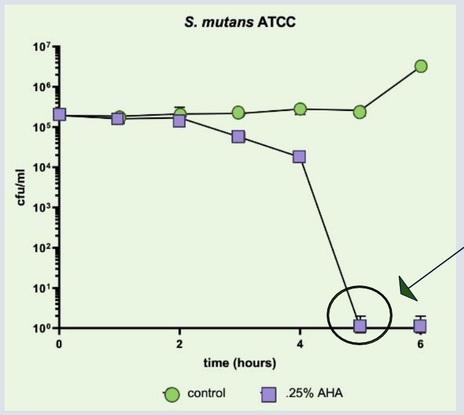
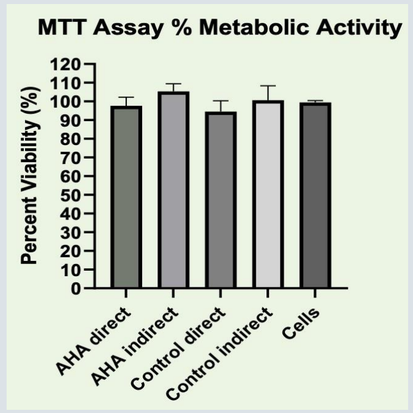


Figure 2. A concentration of 0.25 wt% of AHA within the RPD formulation was able to eliminate 10^5 Colony Forming Units (CFU) of *Streptococcus mutans* at 5 hours of exposure in 500 μL BHI media in comparison with control

Figure 3. No statistically significant differences were found in the activities of the mouse fibroblasts between 0.25% AHA samples and the controls.



Sample	Av. Modulus (GPa)	Av. Flex Strength (MPa)	Redox Conversion (%)	Thermal Post-Cure Conversion (%)
Control - dry	1.59 ± 0.08	79.1 ± 6.0	89.6 ± 1.2	97.4 ± 0.62
.25% AHA - dry	1.57 ± 0.14	70.9 ± 3.9	91.4 ± 1.7	97.2 ± 0.60
Control - wet	1.32 ± 0.09	60.2 ± 5.8	89.6 ± 1.2	97.4 ± 0.62
.25% AHA - wet	1.31 ± 0.09	56.2 ± 3.1	91.4 ± 1.7	97.2 ± 0.60

Figure 4. No significant differences were shown between control and 0.25% AHA modulus, flexural strength, or conversion for 2x2 mm bars of wet and dry samples.

CONCLUSIONS

The inclusion of 0.25 wt% AHA into UDMA:MAA:EHA resin gives the cured polymer *Streptococcus mutans* inhibiting activity. At this concentration of acrylated hydroxyazobenzene, resin polymers are non-toxic to mouse fibroblasts and the addition of AHA does not interfere with conversion or mechanical integrity relative to the control properties in a statistically significant manner.

FUTURE DIRECTIONS

Future work includes assessing the impact of the 0.25 wt% concentration of AHA on the coloring of the denture base with the inclusion of gingiva pigment, determining the effect of AHA on fungal agents and other bacterial strains, producing replicates of the kill curve experimentations, and utilizing TPO photoinitiator and Inkjet UV lamp for the curing process prior to actual jetting. In coordination with dental clinical faculty at the CU School of Dental Medicine, the AHA-modified RPD base will be used in multimaterial inkjet printing of monolithic RPD base and tooth prostheses for eventual use in case studies and clinical trials following appropriate clearances.

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