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

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Streptococcus mutans is a gram-positive coccus bacteria in the oral cavity that contributes to tooth decay. Dentures can harbor *S. mutans,* causing high rates of oral cavity infections among their wearers. Acrylated hydroxyazobenzene (AHA) in (meth)acrylate polymers has shown an inhibitory effect on *S. mutans* at a concentration of 2 wt%. Despite advances in 3D printing and dentistry, the direct fabrication of robust components of full/partial dentures remains an unmet goal. The shift in fabrication methods to 3D printing opens the door to lower cost and rapid production of removable partial dentures (RPDs) for these patients. The objective of this project is to formulate a polymer for a 3D printed RPD base, with appropriate flexural modulus and elasticity, containing AHA for inhibition of caries causing *Streptococcus mutans.*

An RPD resin of 46% urethane dimethacrylate, 37% ethylhexyl acrylate, and 17% methacrylic acid by weight was formulated as the control. To the resin, different concentrations of AHA, 0.25 wt.%, 0.5 wt%, and 1 wt% were added and 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 as thermal initiator). Samples were then subjected to 3-point bend testing under both dry and wet (DI H_2O at 37°C bath for 48 h) conditions to measure flexural strength and modulus. Bacterial kill curves were performed with exposure to the polymer to assess the susceptibility of *S. mutans* to various levels of AHA. MTT cytotoxicity assays were conducted to evaluate the biocompatibility of the polymer.

At a concentration of 0.25 wt% AHA in resin, 100% inhibition of a 10^5 cfu/mL starting culture was demonstrated at 5 hours. At this concentration of AHA, there were no significant differences between the double-bond conversion and mechanical properties between the control and AHA-containing polymers. The RPD and the RPD + AHA polymers showed comparable double-bond conversion (RPD=97.4 $\pm$ 0.6% vs. RPD+AHA=97.2 $\pm$ 0.6%). Mechanical properties were also comparable with modulus (GPa) (RPD=1.59$\pm$ 0.08 vs. RPD+AHA=1.57$\pm$ 0.14) and flexural strength (MPa) (RPD=79.1$\pm$6.0 vs RPD+AHA=70.9$\pm$3.9), indicating that the RPD+AHA meets the ISO standards for dentures. Exposing mice fibroblast cells to the polymer for 24 h demonstrated no decline in cell viability, suggesting good biocompatibility.

The RPD formulation with a noncontact-based *Streptococci*-specific inhibitory additive maintains its high strength and flexibility attributes at a low concentration of 0.25 wt% of AHA. In the coming months, the genus-specific inhibitory ability of AHA against other pathogenic bacteria such as *S. pneumoniae* will also be studied. In coordination with dental clinical faculty at the CU School of Dental Medicine, the AHA-modified RPD base will be used in multi-material inkjet printing of monolithic RPD bases and tooth prostheses for eventual use in case studies and clinical trials following appropriate clearances.