

**ADVANCED  
MATERIALS**  
INTERFACES

Supporting Information

for *Adv. Mater. Interfaces*, DOI: 10.1002/admi.201902149

Selective Inhibition of *Streptococci* Biofilm Growth via  
a Hydroxylated Azobenzene Coating

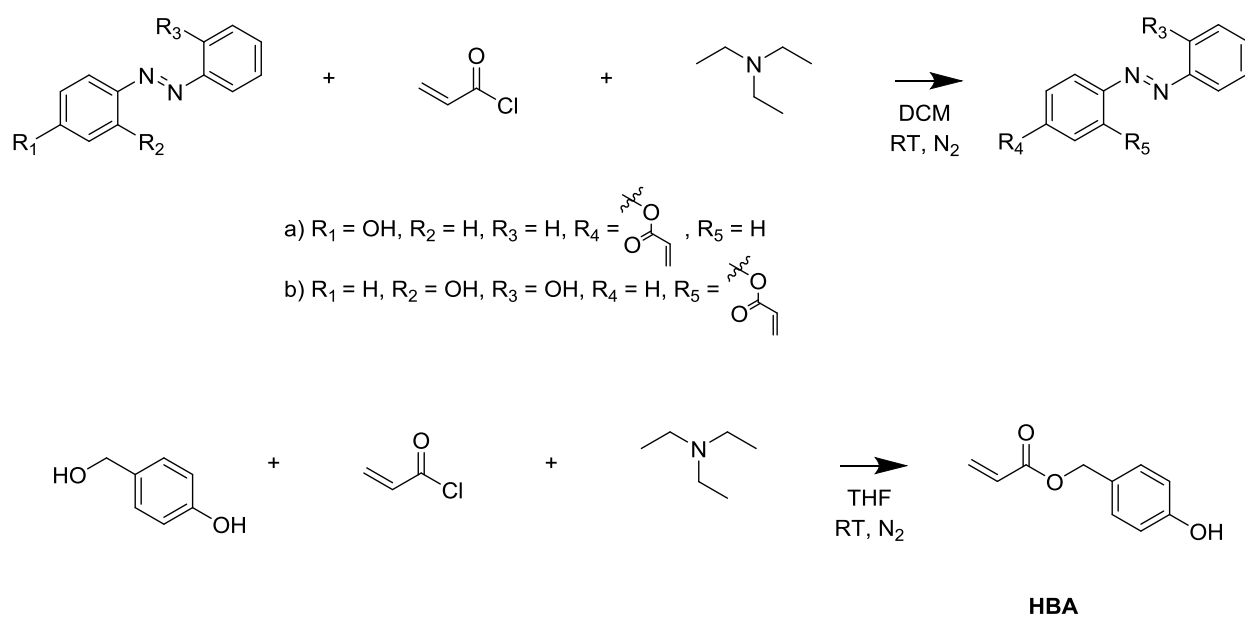
*Dylan I. Mori, Michael J. Schurr, and Devatha P. Nair\**

Copyright WILEY-VCH Verlag GmbH & Co. KGaA, 69469 Weinheim, Germany, 2020.

## Supporting Information

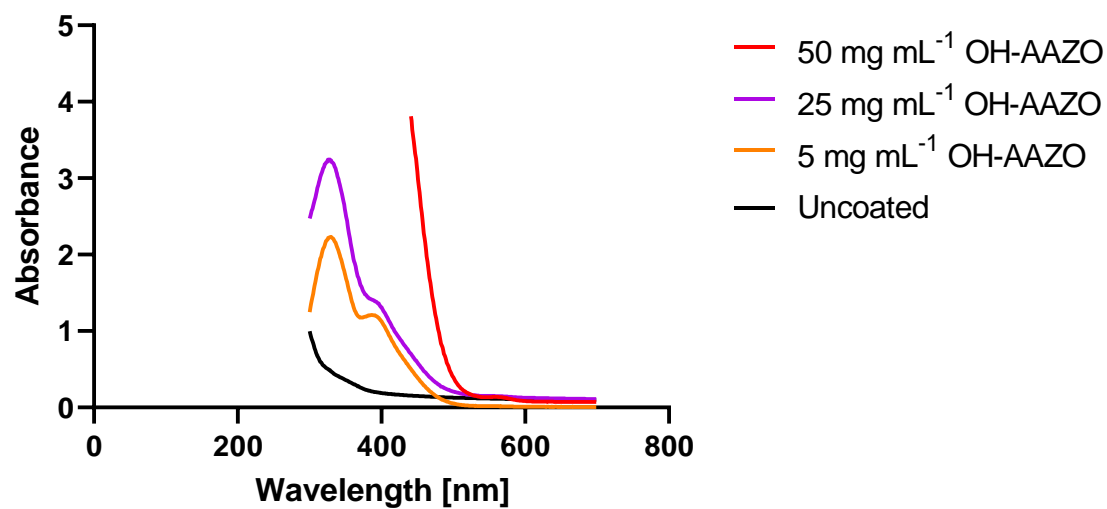
### Selective Inhibition of *Streptococci* Biofilm Growth via a Hydroxylated Azobenzene Coating

Dylan I. Mori, Michael J. Schurr, and Devatha P. Nair\*



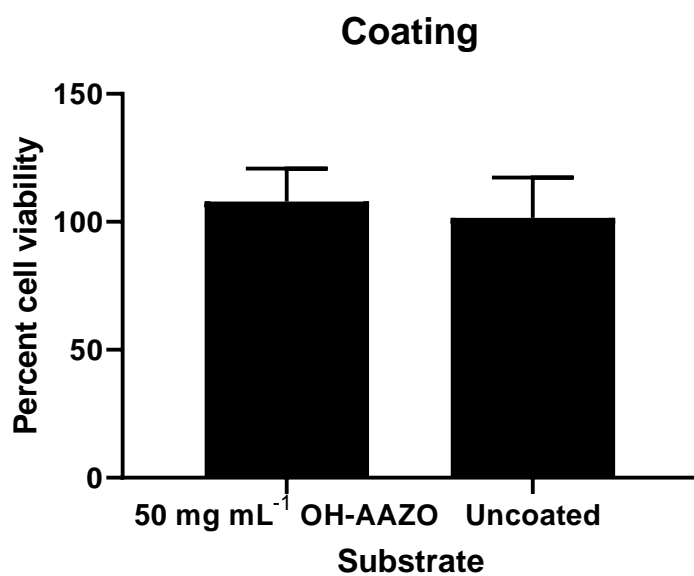
**Figure S1.** Synthetic scheme of an acrylated azobenzene (AAZO, 1a), phenolic acrylated azobenzene (OH-AAZO, 1b), and 4-hydroxybenzyl acrylate (HBA) as a control monomer.

UV-Vis spectra



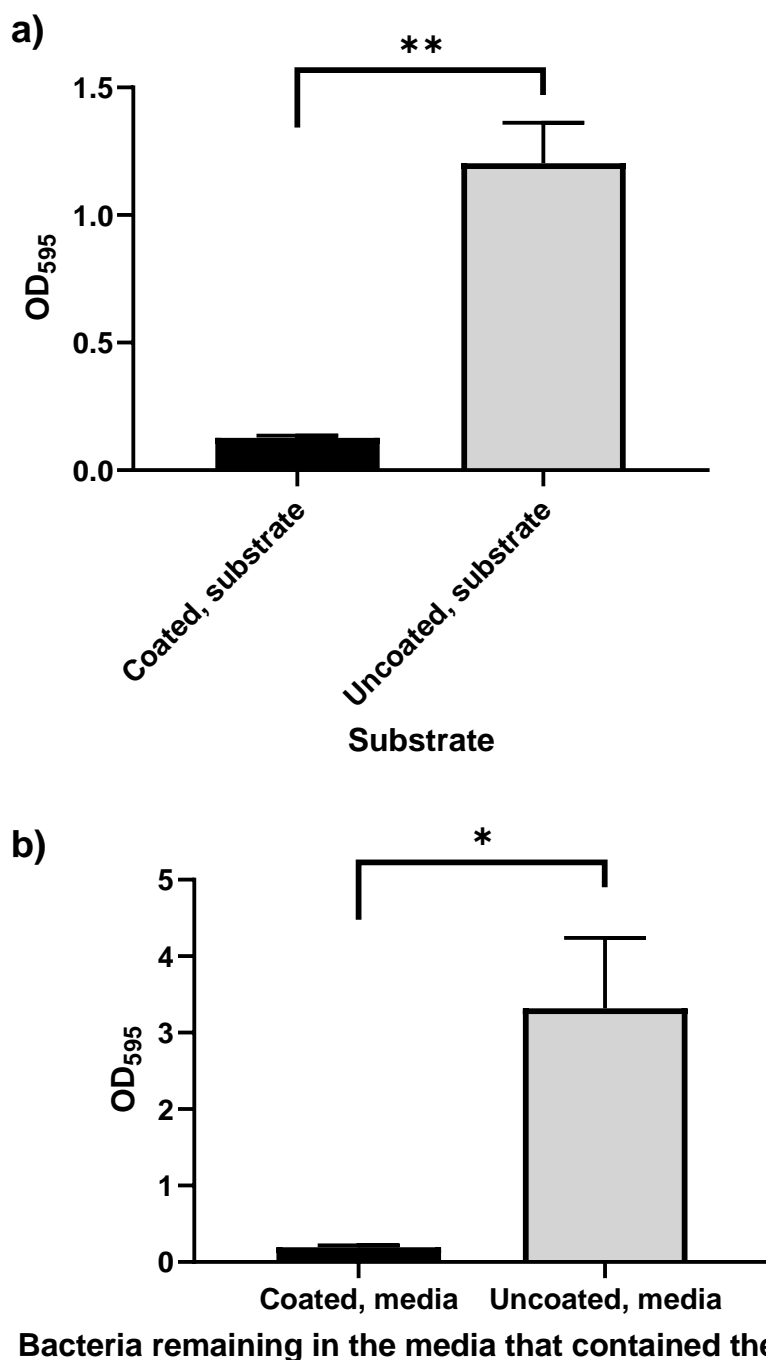
**Figure S2.** Example UV-Vis spectra of OH-AAZO coatings at different concentrations (plus an uncoated control substrate) immediately following thermal curing and aqueous extraction.

Cytotoxicity results



**Figure S3.** Cell cytotoxicity results for L929 mouse epithelial cells on coated 50 mg mL<sup>-1</sup> OH-AAZO substrates. All values were normalized to a positive control (cells grown in the absence of a substrate).

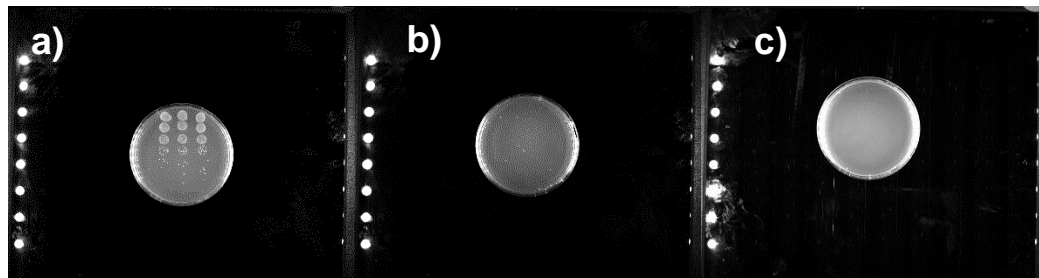
Crystal violet assay



**Figure S4.** Crystal violet assay of *S. mutans* biofilms grown on OH-AAZO-coated and uncoated substrates. After 24 h of biofilm growth, the substrates were removed from the wells, stained with crystal violet solution, and the optical density values were quantified via a plate reader at  $\lambda=595$  nm (S4a). The biofilm remaining in the wells that previously contained the substrates was also stained and quantified using a similar method (S4b). These results reflect the trend observed with the sonication and showed significantly more *S. mutans*

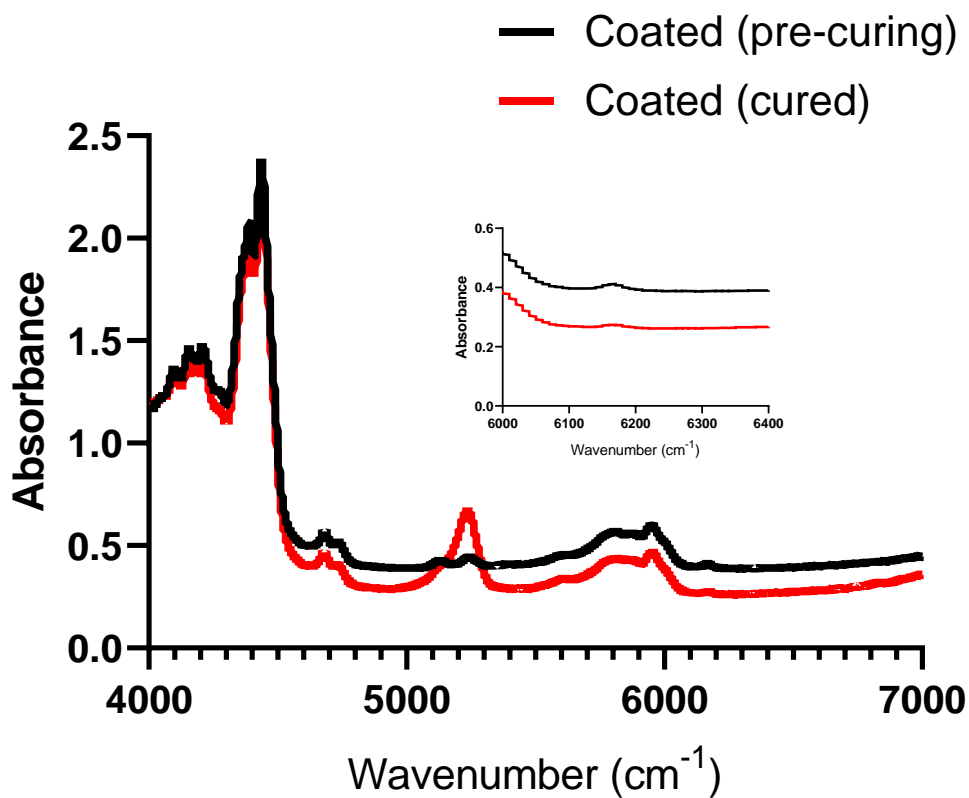
biofilm growth for the uncoated substrates (sonication and media values) relative to the OH-AAZO-coated substrates (n=3 for all, \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , t-test)

Plating of media to confirm bacteria death



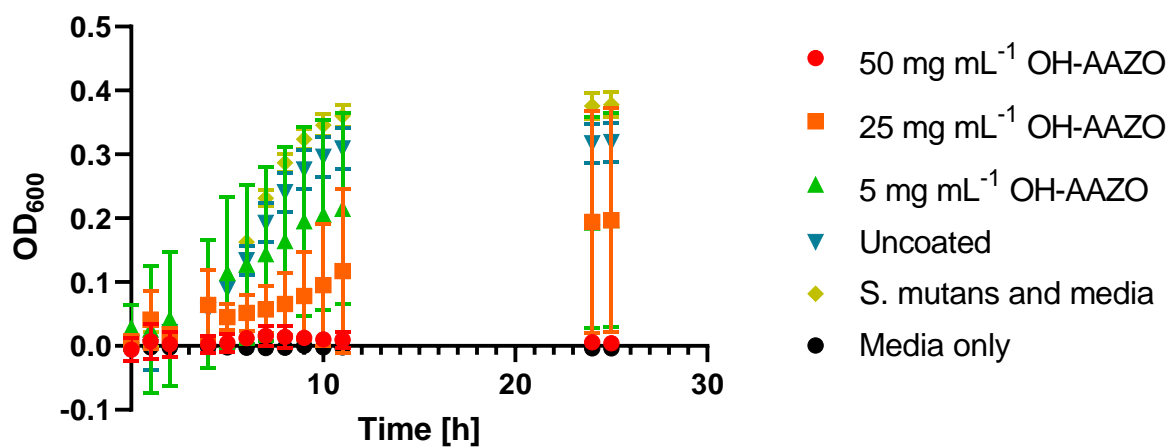
**Figure S5.** Complete plating of media of *S. mutans* in contact with OH-AAZO-coated substrates at different timepoints following seeding. The media sampled in S5a at  $t = 0$  h was serial diluted as described previously and gave a total CFU count of  $(8.0 \pm 3.2) \times 10^6$  CFUs. The media sampled in S5b at  $t = 4$  h was streaked across the entire plate and gave a total CFU count of 8 CFUs. The media sampled in S5c at  $t = 24$  h was streaked across the entire plate and gave a total CFU count of 0 CFUs.

## Curing Study



**Figure S6.** Near FT-IR spectra to monitor the disappearance of the acrylate peak (6260 – 6096  $\text{cm}^{-1}$ ), prior to curing (black) and after curing/extraction (red).

## Growth curves



**Figure S7.** Growth curve data of *S. mutans* in a 96-well plate in the presence of coated OH-AAZO substrates at different concentrations.



**Table S1.** Diameter of kill zone of sampled media over time

Time [h] <sup>a)</sup>	d (1) [mm]	d (2) [mm]	d (3) [mm]	d (LF) [mm]	d (U) [mm]	d (W) [mm]
2	0	0	0	41	0	0
4	0	0	0	45	0	0
6	0	0	0	45	0	0
8	0	0	0	44	0	0
12	0	0	0	49	0	0

*d* = diameter of zone of inhibition for three (3) different samplings of media wells containing OH-AAZO substrates and one uncoated (U) well, LF = LevoFloxacin (positive control, 2.5 mg mL<sup>-1</sup> solution), sterile MilliQ water (W) (negative control). No leaching of OH-AAZO monomer was detected.