Supporting Information

Optically Responsive, Smart Anti-Bacterial Coatings via the Photofluidization of Azobenzenes

Gannon M. Kehe [1], Dylan I Mori, [1] Michael J. Schurr [2] and Devatha P. Nair* [1,3]

[1] Department of Craniofacial Biology, University of Colorado-School of Dental Medicine, Aurora, CO 80045
[2] Department of Immunology and Microbiology, University of Colorado-School of Medicine, Aurora, CO 80045
[3] Materials Science and Engineering, University of Colorado-Boulder, Boulder, CO 80309

Corresponding Author

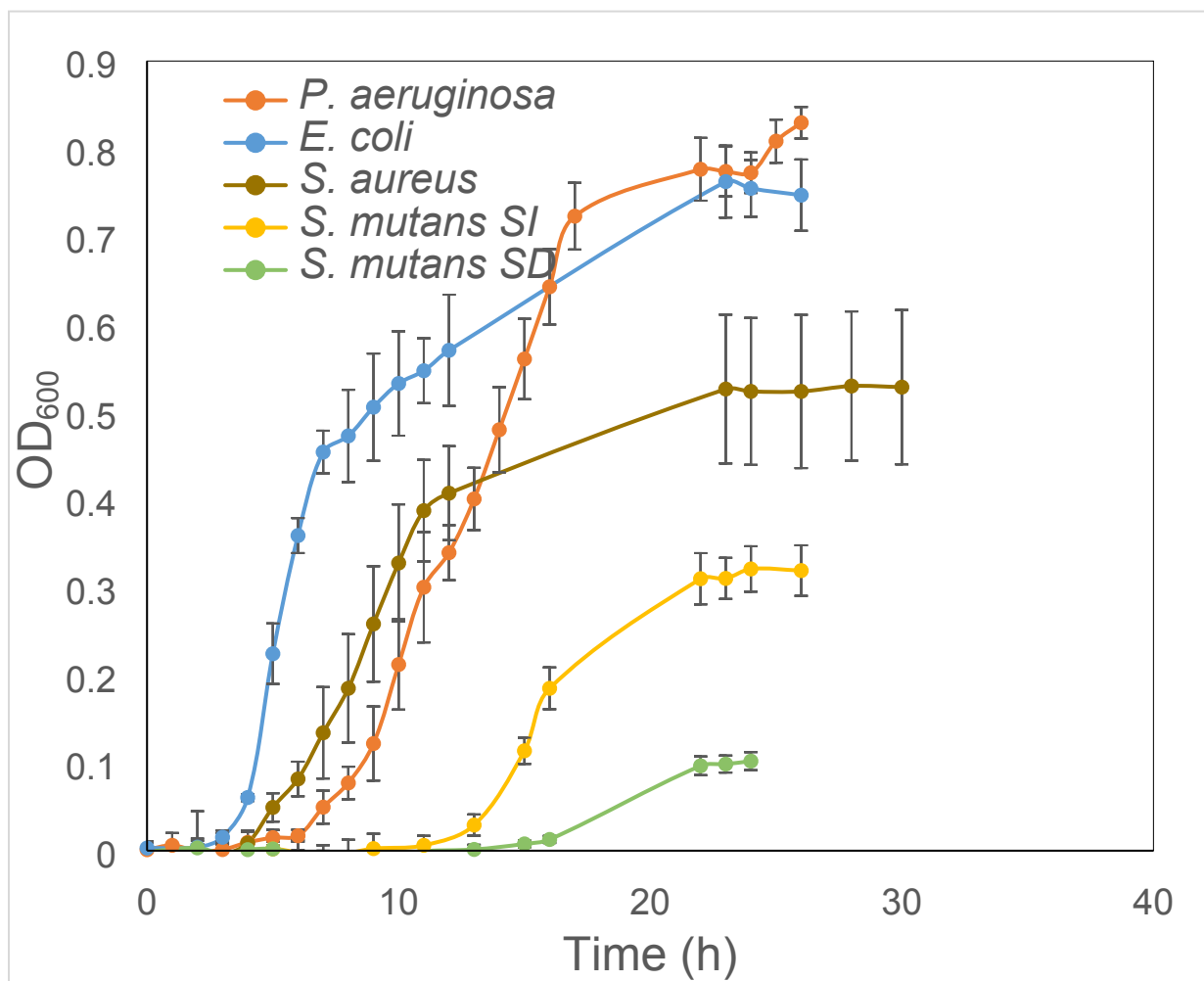*E-mail: Devatha.Nair@ucdenver.edu

Growth curves

Figure S1. Growth curve of bacteria cultures on AZO-coated substrates. The bacteria was grown and individual colonies were inoculated in 5 mL BHI media and incubated in liquid cultures. The stock solution was diluted to 1:10^4 in the media and seeded on the substrates in a 96-well plate. Optical density was monitored at λ = 600 nm. Bacterial strains studied were *P. aeruginosa*, *E. coli*, *S. aureus*, *S. mutans* (both in the presence and absence of sucrose)

Table S1.

Biofilm formation on the substrates

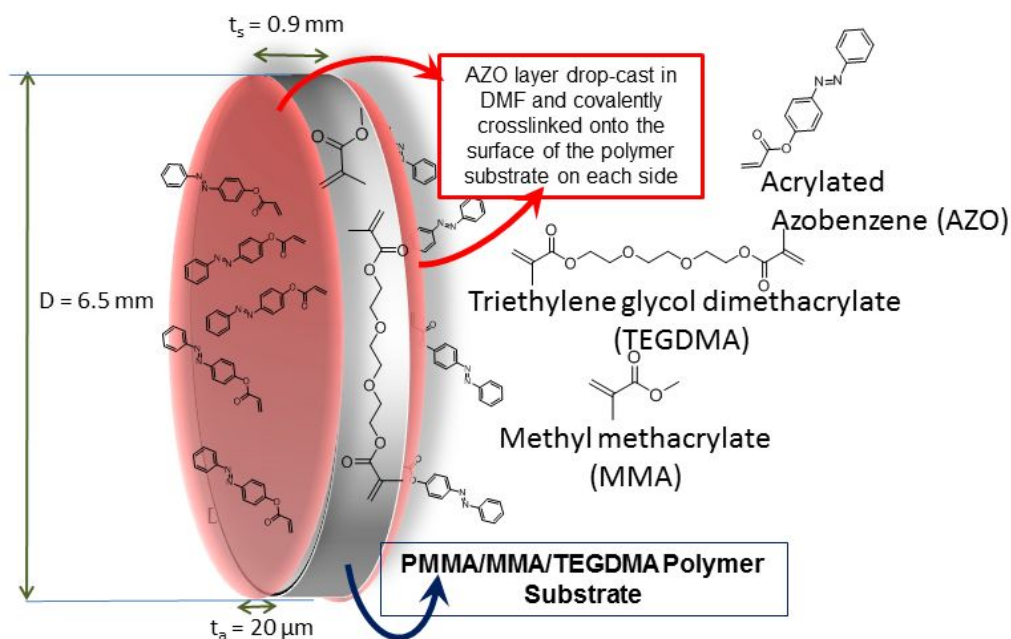| Organism	Growth Time for Biofilm Formation (h)
P. aeruginosa	20
E. coli	24
S. aureus	24
S. mutans-SI	24
S. mutans-SD	24

Sample Fabrication

Figure S2. To formulate the azocoated substrates, acrylated azobenzene (AZO) in DMF is drop-cast onto a glassy polymer substrate of PMMA/MMA/TEGDMA and thermally cured in the presence of Azobisisobutyronitrile(AIBN) t_a -> thickness of the AZO coating, t_s->thickness of polymer substrate.

NMR

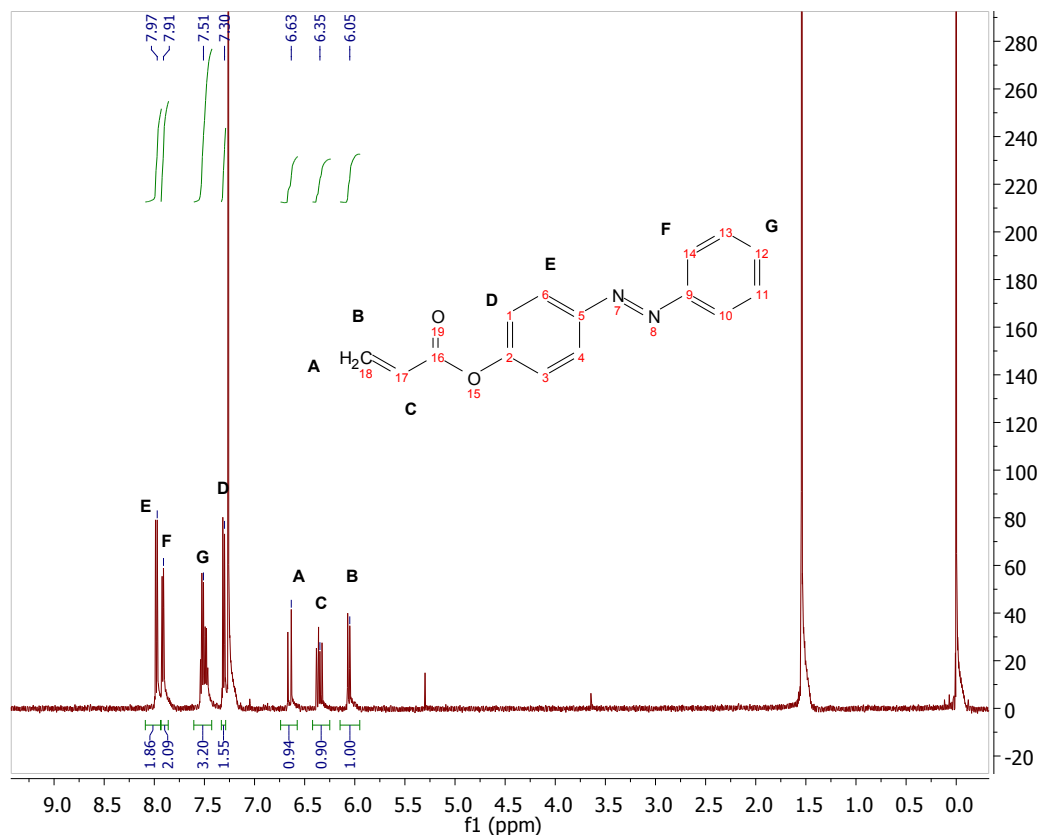**Figure S3.** ^{1}H NMR spectrum of AAZO monomer in CDCl$_3$. ^{1}H NMR: (500 MHz, CDCl$_3$) δ 7.97 (d, 2H), 7.91 (d, 2H), 7.51 (m, 3H), 7.30 (d, 2H), 6.63 (d, 1H), 6.35 (dd, 1H), 6.05 (d, 1H).
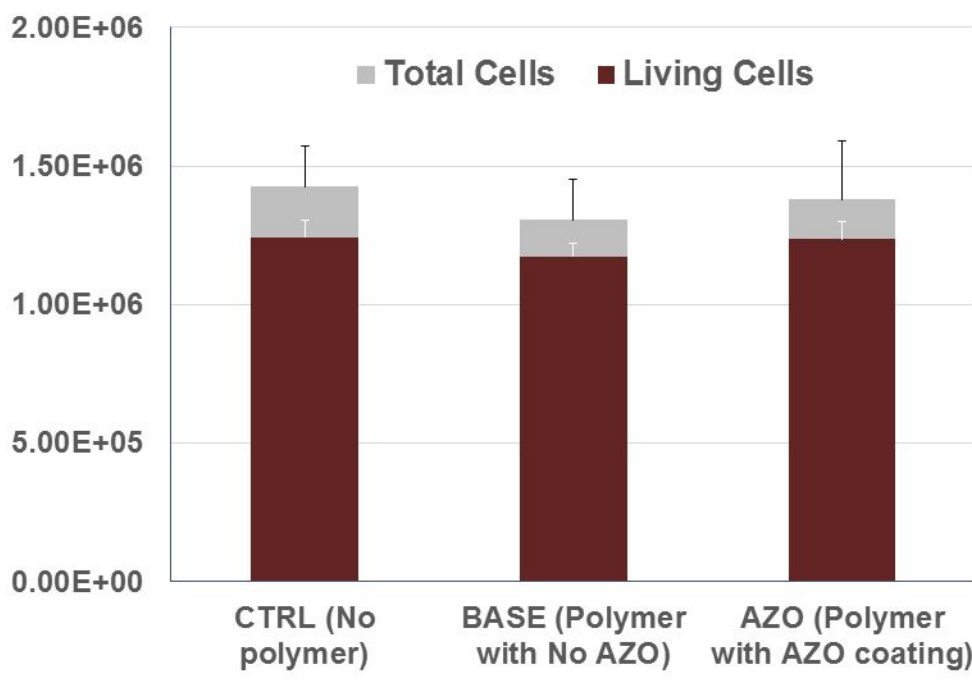
Cytotoxicity Study

Figure S4. *Cytotoxicity Studies.* The cytocompatibility of AZO-coated polymer substrates were evaluated by Trypan blue (ThermoFisher Scientific™) exclusion method for cell viability. Briefly, 50,000 of L929 cells were seeded into six-well tissue culture plates. Cells were fed with minimum essential medium (MEM) supplemented with 10% fetal bovine serum and incubated at 37 °C in 5% carbon dioxide atmosphere for 24 h. Cells were incubated with polymer films at 37 °C for 48 h. The cultured cells were examined microscopically for cellular response using a phase contrast inverted microscope (Leica, WLD MPS32, Germany). The cell viability was quantified via a hemocytometer.

CFU Quantification Protocol

Substrates with mature biofilms are subjected to 5 steps in the absence of ambient light; 2 min initial washes in 10 mL of 1x PBS, 40 s Light Exposure with 3M Elipar™ (20s per side) and 30 s wash in 5 mL of PBS (performed 3X), then sonicated in 5 mL of 1x PBS. The Light Exposure step is repeated 3 times per sample (and the number of samples n ≥ 3 for each test condition). Subsequently, 200 μL of each 1x PBS washing solution is removed and a series of serial 1:10 dilutions is performed on each respective wash sample in a 96-well plate to estimate the biofilm removed in each light exposure/wash step. The associated dilutions for each treatment are then seeded at a volume of 10 μL on BHI-Agar plates (3X) and the plates are placed in a 37℃ incubator for 20 h. Once CFUs have reached a countable size, counts are taken at their respective dilutions and the total amount of bacteria removed in each treatment may be calculated via the following equation

$$\frac{Total\ Amount\ of\ Bacteria\ Removed}{per\ Treatment} = \frac{(CFU\ Count)}{10\ \mu L} * (PBS\ Wash\ Volume) * 10^{Number\ of\ Dilutions}$$

Where, $PBS\ Wash\ Volume = 10{,}000\ \mu L$ for Initial Wash and

$PBS\ Wash\ Volume = 5{,}000\ \mu L$ for Light Exposure and Wash treatments

$PBS\ Wash\ Volume = 5{,}000\ \mu L$ for Sonication Treatment

Statistical analysis

Welch's t-test (p < 0.05) was conducted on the samples and indicated a statistically significant difference between the AZO (Light Exposure) and No AZO (Light Exposure) as well as the AZO (Light Exposure) and No AZO (No Light) in all bacterial strains studied with the exception of *S. mutans*-SD.

Live-Dead Staining Protocol

The live-dead stain was prepared using a two component mixture developed by Invitrogen BacLight™ (ThermoFisher Scientific™) by adding 1.5 μL of component A (SYTO® 9, green fluorescent nucleic acid stain) and component B (propidium iodide, red fluorescent nucleic acid stain) to 200 μL of sterile water. Samples were subjected to light exposures/washes as previously described, then soaked in the stain solution for 3 min and gently rinsed in sterile water for 10 s. Substrates are then fixed to a glass slide and imaged under a (Zeiss) digital microscope using FITC and CY3 channels.

TABLE S2.

List of Strains Used in This Study

Strain	Relevant properties	Reference or origin
P. aeruginosa	PAO1	{Holloway, 1955}
E. coli	UTI89 Uropathogenic	{Mysorekar, 2013}
S. aureus	Methicillin-susceptible *Staphylococcus aureus*	{Herbert, 2010 HG001 or AH2183 or RN1HG}
S. mutans	*Streptococcus mutans*	ATCC 25175

References

Holloway, B. Genetic recombination in *Pseudomonas aeruginosa*. *J Gen Microbiol* **1955**, 13,572-581.

Wang,C.; Symington, J.; Ma, E.; Cao, B.; Mysorekar, I.Estrogenic modulation of uropathogenic *Escherichia coli* infection pathogenesis in a murine menopause model. *Infect. Immun.* **2013**, 81,733–739

Herbert, S.; Ziebandt, A.; Ohlsen, T.; Hecker, M.; Albrecht, D.; Novick, R.; Götz, F. Repair of global regulators in *Staphylococcus aureus* 8325 and comparative analysis with other clinical isolates *Infect. Immun.* **2010**, 78,. 2877–2889.