

## Demonstration of Microfluidics for Fluorescence-Based Pathogen Detection Assays using a Thiol-Acrylate Resin

W. Zhang,<sup>a,b</sup> M. Tullier,<sup>c</sup> K. Patel,<sup>a,b</sup> A. Carranza,<sup>c</sup> J. A. Pojman<sup>c</sup> and A. D. Radadia<sup>a,b</sup>

<sup>a</sup> Institute for Micromanufacturing, Louisiana Tech University, Ruston, Louisiana 71272, United States.

<sup>b</sup> Center for Biomedical Engineering and Rehabilitation Sciences, Louisiana Tech University, Ruston, Louisiana 71272, United States.

<sup>c</sup> Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803, United States.

### SUPPORTING INFORMATION

#### MATERIALS AND METHODS

**Materials:** All stock solutions were prepared using deionized water (min. resistivity of 18.0 MΩ-cm) obtained from a Millipore deionized water system. Pentaerythritol triacrylate (PETA) stabilized with 300-400 ppm 4-methoxyphenol was obtained from Alfa Aesar. Trimethylolpropane tris(3-mercaptopropionate) (TMPTMP, ≥95%) was obtained from Evans Chemetics LP. Diethylamine (DEA, ≥99.5%), and casein blocking buffer (10X) was obtained from Sigma Aldrich. A Sylgard 184 kit was purchased from Elsworth Adhesives to prepare cured PDMS. Phosphate-buffered solution (PBS) and PBS with Tween 20 (PBS-T20) were bought from Amresco, Inc in powder form and reconstituted to form working PBS and PBS-T20 solutions. Antibodies to *E. coli* O157:H7, unlabeled and labeled with fluorescein isothiocyanate (FITC) were procured from Kirkegaard & Perry Laboratories. Lipopolysaccharides from *E. coli* O55:B5 conjugated with Alexa Fluor® 568 dye, and polymyxin B labeled with BODIPY FL were obtained from Life Technologies.

**Procedure to make the devices:** DEA was added to PETA in a ratio of 16.1 mol% DEA based on acrylate groups (%DEA = (mol DEA)/(mol acrylate groups + mol DEA)). The two were mixed thoroughly and then stirred for at least three hours at room temperature for the reaction to proceed to completion. To make the microfluidic devices, the above solution was mixed with TMPTMP and stirred well. To remove bubbles from this mixture prior to curing, the mixture was centrifuged at 5000 rpm for 3

min. Two types of substrates were cured, one with channels and the other one flat. For the side with the channels, a mixture with 40% excess PETA moles compared to moles TMPTMP was made and poured over a machined PMMA mold in a plastic petri dish. For the flat side, a mixture with 40% excess TMPTMP moles compared to moles PETA was used and poured directly into a plastic petri dish. After 1 hour of curing at room temperature, the cured thiol-acrylate substrates were pulled out from their molds. Holes were drilled into the channel side of the chip via a Dremel drill press with a general purpose 1/16" bit. Any particulate matter generated from the drilling process of the holes was washed out with ethanol and blow dried with nitrogen. The two halves of the microfluidics were aligned and pressed together. Air bubbles trapped between the pieces were pressed out by hand, and the assembly was bonded by placing it under a load of ~1.4 kg/square inch for 24 hours.

**Preparation of PDMS:** The curing agent was mixed with the silicone elastomer base in 1:10 ratio on a weight basis, mixed thoroughly using a stirrer, poured into a petri dish, degassed for 30 min at 24 inch Hg and cured on a hot plate at 55 °C for 4 hours.

**Fluorescent labeling of *E. coli* O157:H7 bacteria:** The live culture of *E. coli* O157:H7 was created by inoculating in Luria-Bertani broth (LB) at 37 °C for 12 hours. Based on agar plating of the culture at the 12<sup>th</sup> hour, all bacteria were found to reach a stationary phase of growth with an average 10<sup>9</sup> colony forming units per mL (cfu/mL). Live culture was used for work in this paper. The culture (1 mL) was washed with phosphate saline buffer (PBS) via centrifuging at 12k rpm for 2 minutes, followed by replacement of 0.9 mL of the supernatant with PBS and resuspension of the bacterial pellet via vortex mixing. The washing procedure was done two times. TAMRA-SE (2 µl, 5 mg/mL) was mixed into the washed culture and incubated at 37 °C for 30 minutes. Excess labeling dye was removed by washing with PBS four times as described earlier.

**Functionalization of acrylate-rich microfluidic channel with proteins:** The tubing was pretreated with casein blocking agent for 1 hour and rinsed with PBS prior to use with the microfluidic device. Tubing with 1/16" OD was found to fit the access holes drilled in the thiol-acrylate pieces. The channels were washed using 1 mL DI water, followed by functionalization with *E. coli O157:H7* antibody (50 µg/mL) or Polymyxin B (100 µg/mL) by filling up the channels and incubation for 16 hours at 4 °C. The unbound antibodies were washed by flowing 2 mL PBS-T20 at 10 µL/min followed by 1 mL PBS at 10 µL/min. The channel was then filled with casein blocking agent and incubated for 1 hour at room temperature. The washing routine described prior with PBS-T20 and PBS was repeated to remove loosely bound casein.

**Bacteria capture in antibody-functionalized microfluidic channel:** TAMRA-SE labeled *E. coli O157:H7* was diluted to  $10^5$ ,  $10^6$  and  $10^7$  cfu/mL and 1 mL of each was pumped at 10 µL/min through the microfluidic channel functionalized with *E. coli O157:H7* antibody to perform the bacteria capture. To remove the non-specifically bound bacteria, the channel was washed with 3 mL PBS at 10 µL/min.

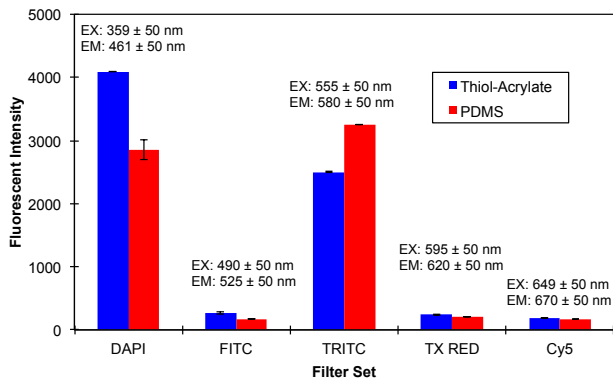
**LPS capture in Polymyxin B functionlized microfluidic channel:** The as-received *E. coli O55:B5* LPS was diluted to 100 µg/mL and 1µg/mL, and 1 mL of LPS solution was pumped through the microfluidic channel functionalized with Polymyxin B at the flow rate of 10 µL/min. The channel was then washed by pumping through 3 mL PBS at the flow rate of 10 µL/min to remove the non-specifically bound LPS.

**Florescence background on thiol-acrylate and PDMS substrates:** The focus of the microscope was set to the upper surface. Images from same location were taken by changing to different filter sets. Images were taken at five different locations. The average intensity was calculated using ImageJ.

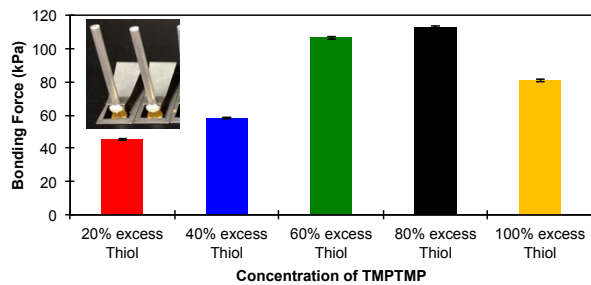
**Bonding force of thiol-acrylate polymer and gold:** The thiol-acrylate polymer was precut to 7 mm diameter cylinders. One flat end of polymeric cylinder was bonded to the 1 cm by 1 cm gold surface coated on a silicon wafer by clipping them together for 24 hours at room temperature. Bonding force was measured using

Sebastian II pull tester. A pre-epoxy coated face of a nail-shaped stud was epoxied to the other circular end of the polymeric cylinder, and then an exact perpendicular controlled rate of force was applied until bonding failure. The data were collected with three readings for each sample.

SUPPLEMENTARY FIGURES



**Figure S1.** Comparison of fluorescence from thiol-acrylate (blue bars) and polydimethylsiloxane surfaces (red bars) using excitation/emission filter pairs for DAPI, FITC, TRITC, Texas Red and Cy5.



**Figure S2.** Bonding force investigation on thiol – gold surface with different concentration of thiol group.