

Supporting Information

Cell resistant zwitterionic polyelectrolyte coating promotes bacterial attachment: An adhesion contradiction

Jessica S. Martinez[†], *Kristopher D. Kelly*[‡], *Yara E. Ghousoub*[‡], *Jose D. Delgado*[‡], *Thomas C.
S. Keller III*[†], and *Joseph B. Schlenoff*^{*‡}

Department of Biological Science and Department of Chemistry and Biochemistry,

Florida State University, Tallahassee, Florida 32306

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* To whom correspondence should be addressed. E-mail: schlen@chem.fsu.edu

[†] Department of Biological Science.

[‡] Department of Chemistry and Biochemistry.

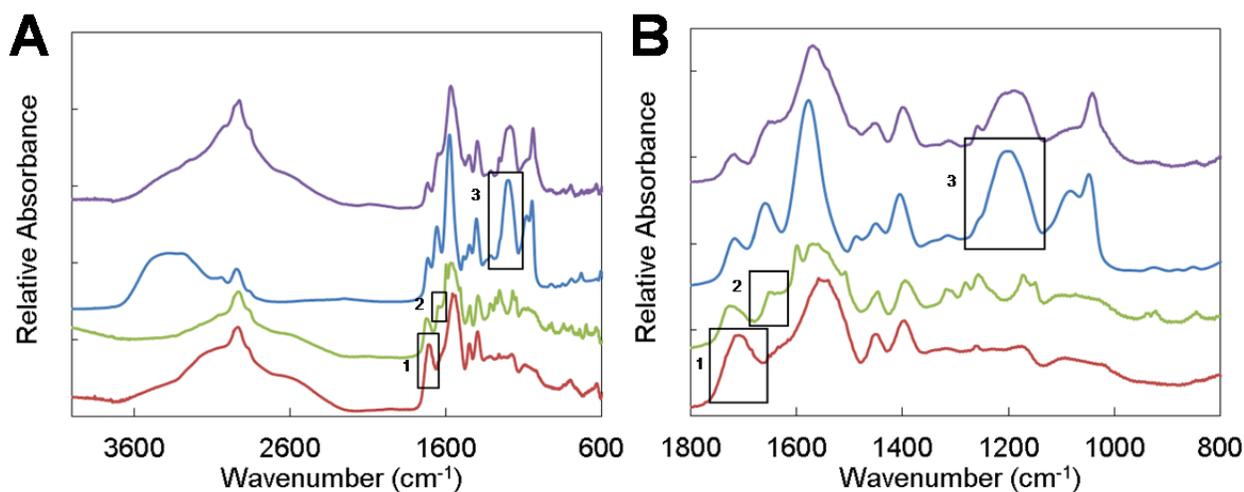


Fig S1. FTIR Spectra of a $(\text{PAH/PAABp})_2\text{-X-(PAH/PAA-co-AEDAPS)}_4$ PEMU and Component Reference Films. Six bilayers of PAH/PAA (red), PAH/PAABp (green), a cast film of PAA-co-AEDAPS (blue), and $(\text{PAH/PAABp})_2\text{-X-(PAH/PAA-co-AEDAPS)}_4$ (purple) are compared via FTIR spectroscopy **A**; and **B** "zoom-in". The numbered boxes represent characteristic peaks of each film with a carboxylic acid stretch from the PAH/PAA film (1), a decomposed diarylketone peak from the post-crosslinked PAH/PAABp film (2), and a sulfonate stretch from the cast film of PAA-co-AEDAPS (3).

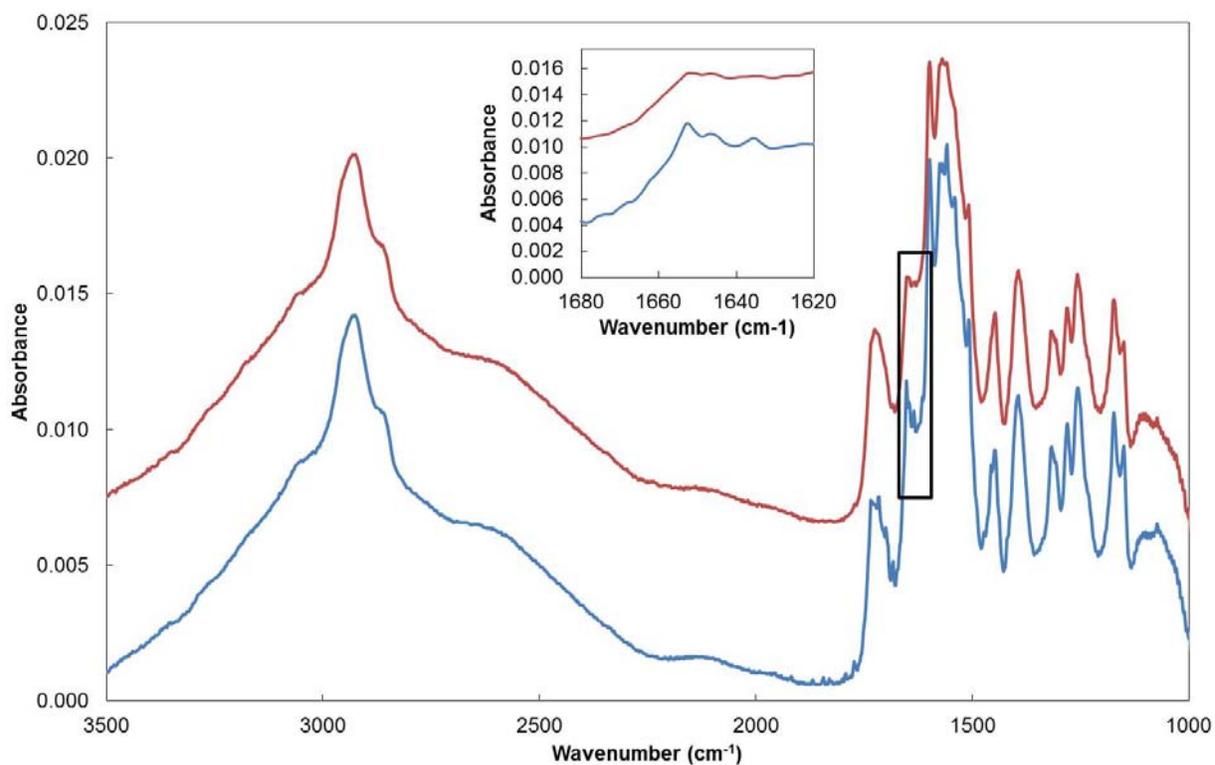


Fig S2. Diarylketone Peak Reduction after UV Exposure of PAABp-containing PEMUs.

FTIR spectrum of $(\text{PAH}/\text{PAABp})_2\text{-}(\text{PAH}/\text{PAA-}co\text{-AEDAPS})_4$ PEMUs (blue) contains a diarylketone peak at $\sim 1650\text{ cm}^{-1}$, which decreases after exposure to UV light between 200-280 nm for 15 min. The PAABp degradation produces free radicals that drive random C-C covalent bond crosslinking within the PEMU and strengthens the film creating the $(\text{PAH}/\text{PAABp})_2\text{-X-}(\text{PAH}/\text{PAA-}co\text{-AEDAPS})_4$ PEMU denoted as **AEDAPS** in this investigation.

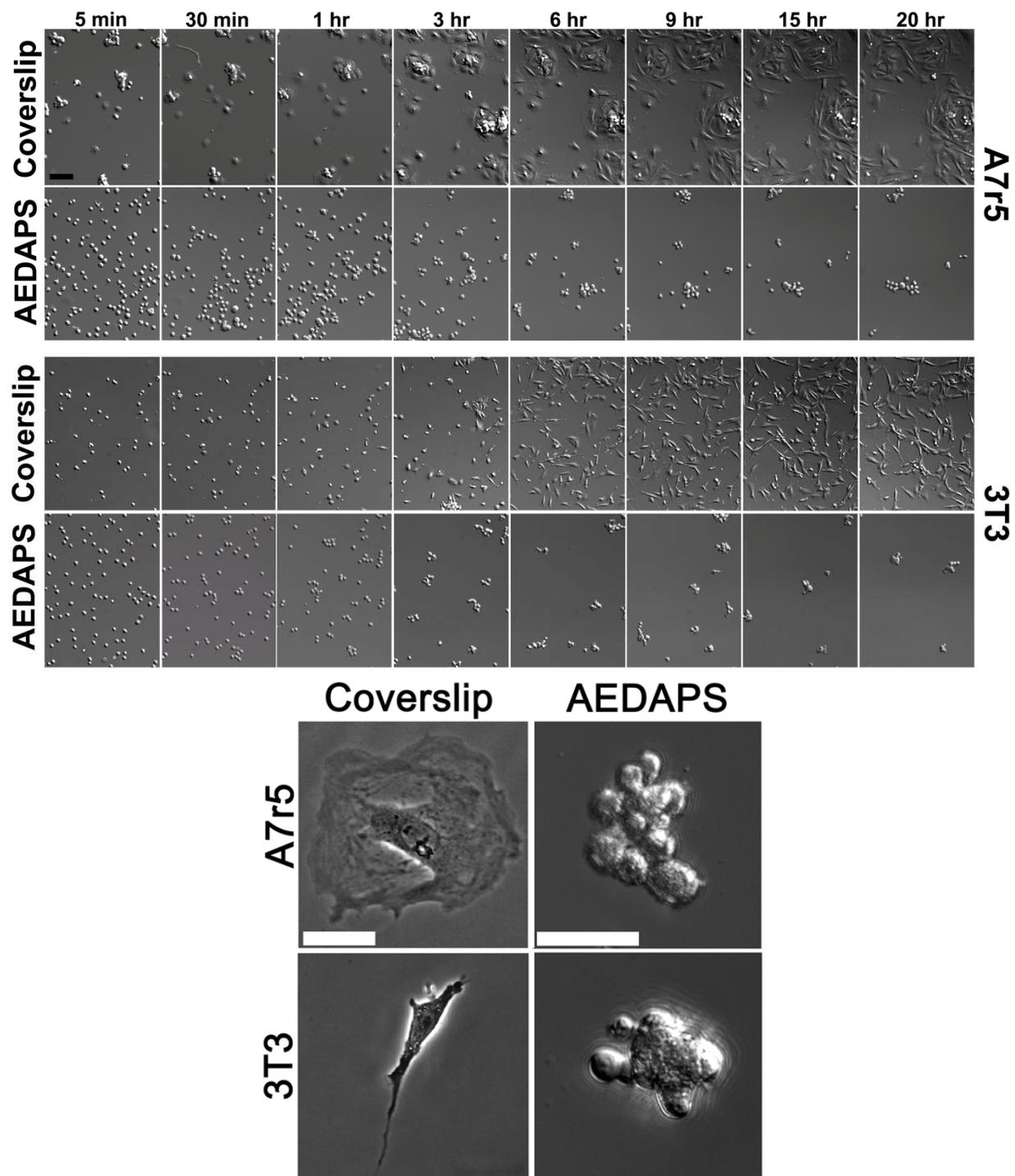


Fig S3. Upper panels: Live A7r5 and 3T3 cells on Uncoated and AEDAPS-Coated Coverslips. Uncoated glass coverslips and **AEDAPS** (PAH/PAABp)₂-X-(PAH/PAA-co-AEDAPS)₄ coated glass coverslips were seeded with A7r5 or 3T3 cells and cultured at 37 °C

with 40% relative humidity and 5% CO₂. Images are taken at 5 and 30 min and 1, 3, 6, 9, 15, and 20 h of culture. Scale bar is 100µm. **Lower panels:** Detail of A7r5 and 3T3 cells on control (bare coverslip) and AEDAPS-coated coverslips after 24 h of culture. Scale bars are 50 µm.

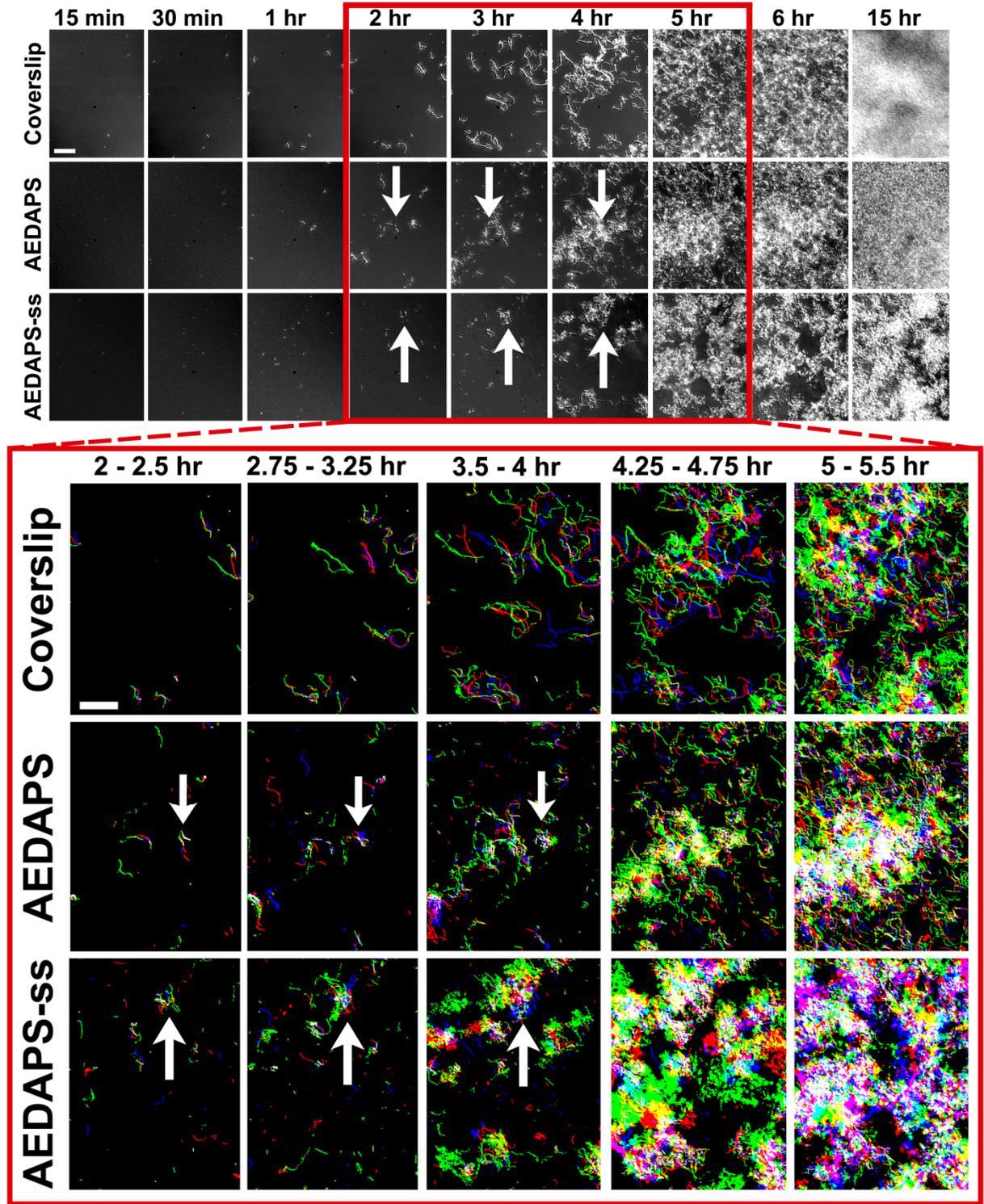


Fig S4. *E. coli* Adhesion over Time on Uncoated, AEDAPS, and AEDAPS-SS Coated Coverslips. Uncoated coverslips and coverslips coated with AEDAPS and AEDAPS-SS

PEMUs were inoculated with 1.5×10^5 CFU *E. coli* in 3 mL media cultured at 37 °C with 40% relative humidity, and imaged at 30 min intervals. The 2 - 5.5 h time frame from (A) is expanded in (B), in which bacteria in three images for each of the time frames indicated were pseudocolored blue (first image), red (15 min image), and green (30 min image) and merged to show bacteria pseudocolored magenta (blue and red overlap indicating immobilization only for the first 15 min time period), yellow (red and green overlap, indicating immobilization only for the second 15 min time period), and white (blue, red, and green overlap, indicating immobilization for the entire 30 min time period). White arrows in (A) and (B) point to regions on the **AEDAPS** and **AEDAPS-SS** surfaces where *E. coli* irreversibly attached and initiated biofilm formation. Scale bar is 50 μm . For details of how merged images were created see Supporting Information, Fig S5. For larger sized image of time lapse recording see Supporting Information, Fig S6.

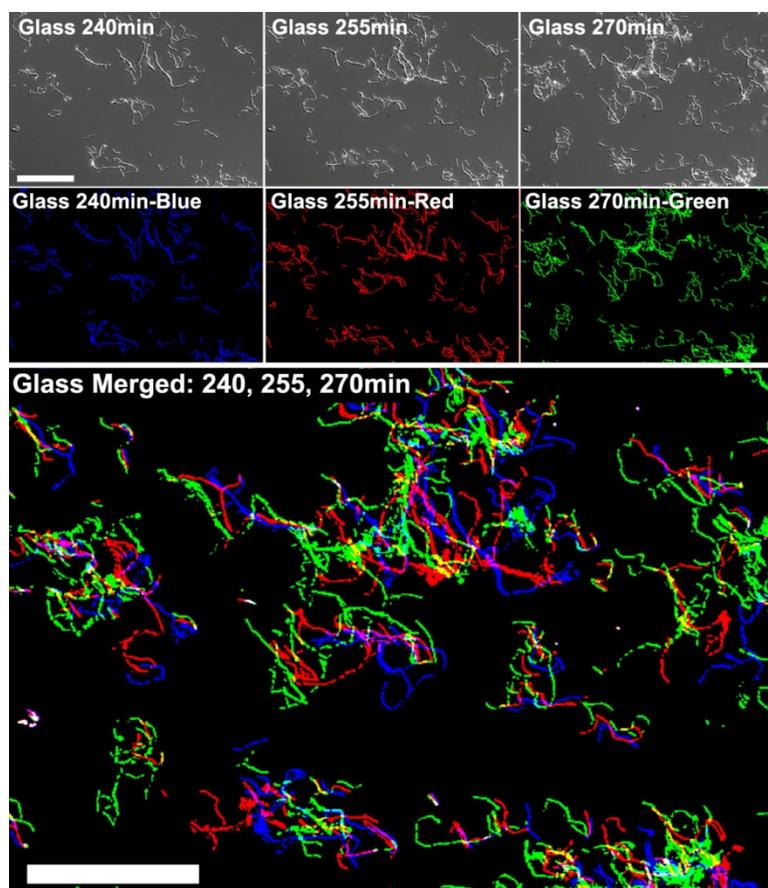


Fig S5. Tracking Time Lapse of *E. coli* on Uncoated Coverslips. Surfaces were inoculated with 5×10^3 *E. coli* CFUs mL^{-1} immersed in 3 mL of Luria Broth media. Live cell imaging was conducted for surfaces immediately after inoculation using a Live Cell™ Chamber (Pathology Devices, Westminster, MD) maintained at 37 °C with 40% relative humidity. Still frames of time lapse recording taken at 240, 255, 270 min after seeding. These three sequential images (at 15 min intervals) were merged together. DIC images (1st row) were converted to binary using ImageJ and pseudo-imposed with colors (2nd row): blue, red, and green. Images were overlaid and merged. Regions where image overlay and produce yellow, magenta, and white colors signify regions of *E. coli* attachment and reduced movement during the three 15 min intervals. Scale bar is 100 μm .

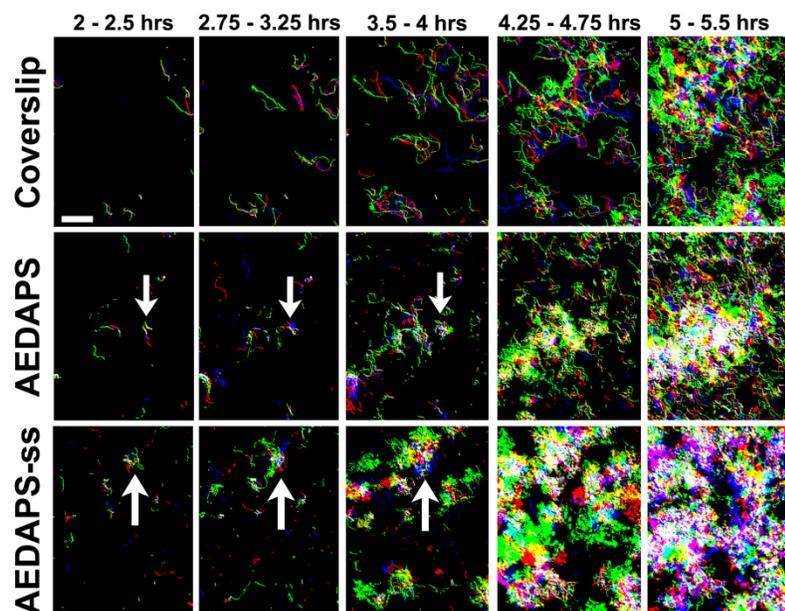


Fig S6. Enlarged Time Lapse of *E. coli* on Uncoated, AEDAPS, and AEDAPS-SS Coated Coverslips. Images of live cells taken at 30 min from 2 to 5 h of culture are shown. Image is enlarged time lapse image from Fig 3 and Fig S4. White arrows point to one of the regions along the **AEDAPS** and **AEDAPS-SS** surfaces where *E. coli* cells irreversibly attached and greatly propagated the initiation of biofilm formation compared to that of uncoated surfaces. Scale bar is 50 μm .

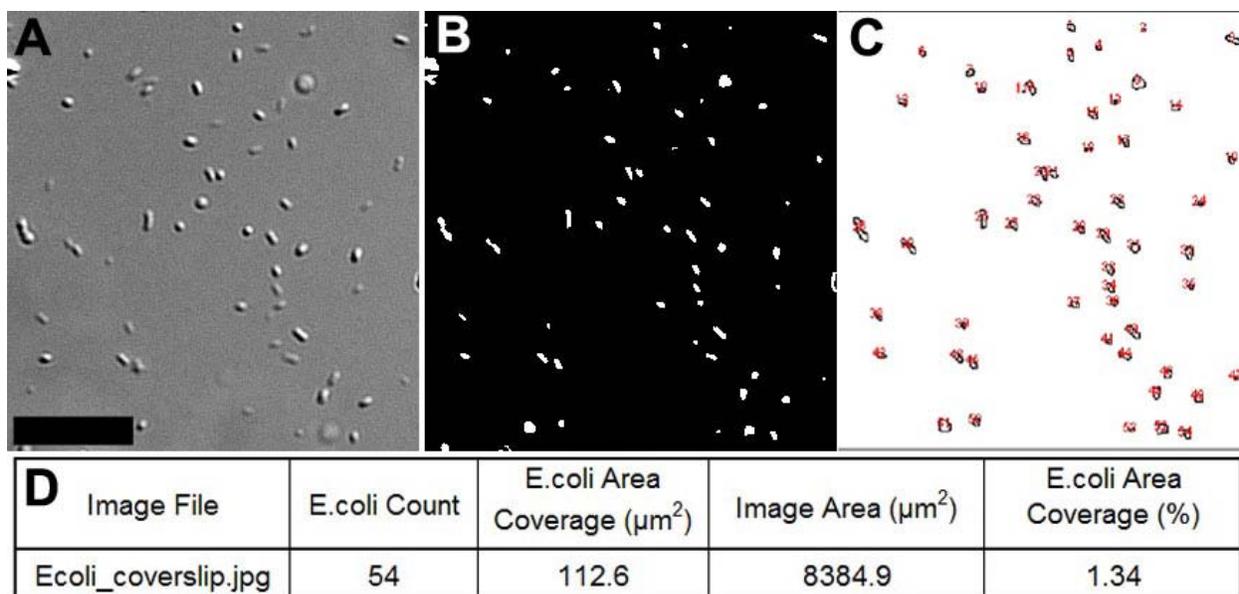


Fig S7. Surface Coverage Percentage ImageJ Analysis of *E. coli* on Uncoated Coverslips.

DIC image of *E. coli* on glass coverslip after 24 h of incubation (A) is converted to a binary image (B) and analyzed using ImageJ (NIH imaging software) (C) and Microsoft Excel (D) to calculate *E. coli* area coverage (total area covered by white pixels) and the *E. coli* area coverage % (total area covered by white pixels compared to total area of the image). Scale Bar 25 μm .

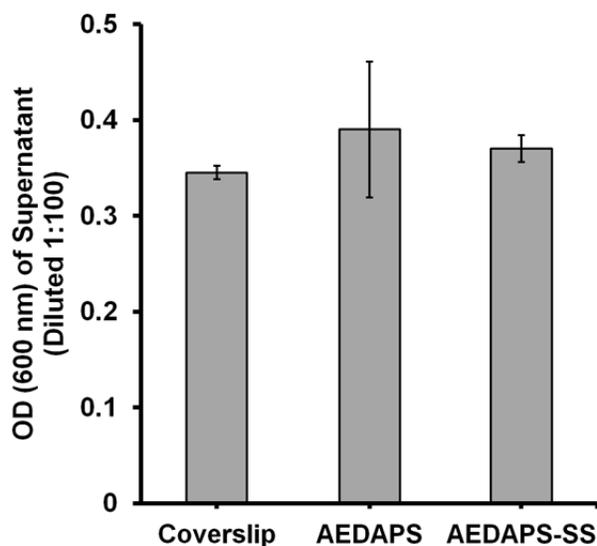


Fig S8. Suspended *E. coli* from Uncoated and PEMU Coated Coverslips for 24 h. Liquid suspension of *E. coli* (inoculate supernatant) were collected after 24 h of static culture incubation at 37 °C for *E. coli* inoculated at 5×10^4 CFUs mL⁻¹ on uncoated coverslip, (PAH/PAABp)₂-X (PAH/PAA-AEDAPS), and (PAH/PAABp)₂-X-(PAH/PAA-AEDAPS)₄ soaked in PAA-co-AEDAPS for 16 h (AEDAPS-SS). Supernatant was diluted 1:100 in PBS and kept on ice to stop further division. OD values were taken at 600 nm. No statistically significant difference was observed between tested surfaces compared to uncoated coverslip. Error bars are standard deviation.

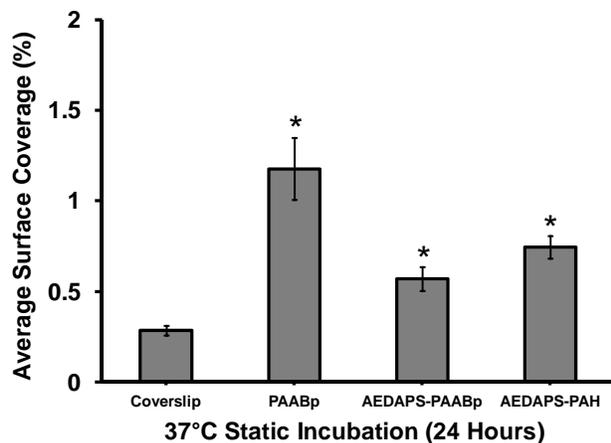


Fig S9. Surface Coverage Percentages of *E. coli* on Glass and PEMUs. Average surface coverage percentages of adherent *E. coli* after 24 h of static incubation with 1.5×10^5 CFUs of *E. coli* on uncoated coverslips and (PAH/PAABp)₆, (PAH/PAABp)₂-X-(PAH/PAA-co-AEDAPS)₄PAABp, and (PAH/PAABp)₂-X-(PAH/PAA-co-AEDAPS)₄PAH coated coverslips. Asterisks (*) indicate Student's T-test P values of <0.05 for significance of difference compared to average surface coverage percentage of uncoated coverslips and other tested surfaces.

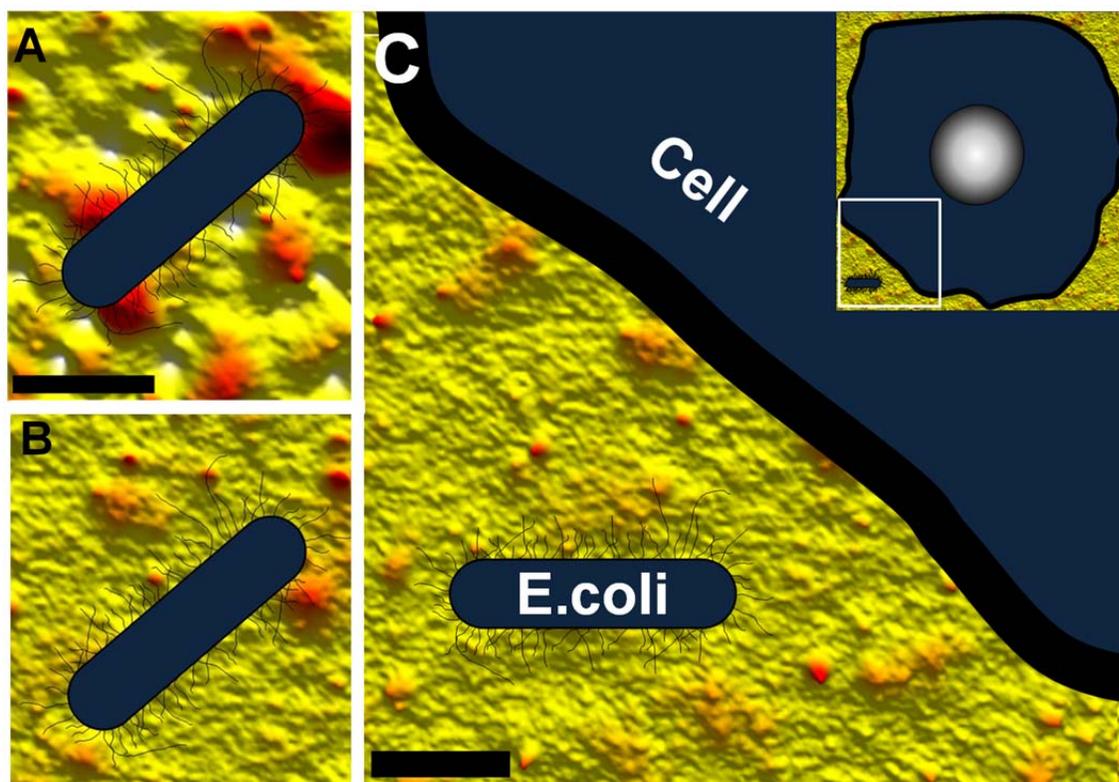


Fig S10. Illustrative Comparison of *E. coli* and Mammalian Cell Attachment on PEMUs.

Cartoon illustration of *E. coli* bacterium is $0.5 \times 2 \mu\text{m}$ in size with fimbriae drawn to scale using an SEM image of *E. coli* K-12 as a reference for proportions and distribution. The *E. coli* cartoon image is overlaid on the $5 \times 5 \mu\text{m}$ AFM image of AEDAPS (A) and AEDAPS-SS (B). A cartoon of a cell ($50 \mu\text{m}$ diameter) with its nucleus (gray, $6 \mu\text{m}$ diameter) is compared to the cartoon *E. coli* (proportions maintained) overlaid on AFM image of AEDAPS-SS (C). Scale bar is $1 \mu\text{m}$.