Electronic Supplementary Material (ESI) for RSC Advances

Electronic Supplementary Information

Accurate and Effective Live Bacteria Microarray Patterning on Thick Polycationic Polymer Layer Co-Patterned with HMDS

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Experimental

Fabrication of polymer patterns:

Glass slides were first cleaned using oxygen plasma (Tegal Plasmaline Photoresist Asher) for 10 min to remove surface organic contaminants. Surface was immediately coated with hexamethyldisilazane (HMDS) by evaporation in HMDS vapor for 10 min. Conventional photolithography process was carried out to generate arrays of 2 μm holes in a positive photoresist (AZ5214E, Clariant Inc.) layer. Next, the developed samples were cleaned again in oxygen plasma for 3 min to etch the HMDS layer and reveal the silanol groups in the exposed holes. Immediately after oxygen plasma, 0.1% (v/v) of polyethyleneimine (PEI; MW50k, Polysciences Inc.) or poly-L-lysine (PLL; MW300k, Sigma) in water was dispensed evenly onto the sample surfaces and incubated for 15min at room temperature in the dark. After incubation, excess polymeric solution was blown away until dry with a stream of nitrogen gas. A lift-off process in acetone with 10 second ultrasonic pulses were used to dissolve the photoresist and lift-off the polymeric layer on it. Samples were finally rinsed with isopropyl-alcohol (IPA) and spin dried. It should be noted that the samples should not be rinsed in water due to its high solubility to the polymers.

Generation of cell patterns:

Wild type *E. coli* strain JCL16 cells were grown overnight in 3 mL Luria-Bertani (LB) broth in a rotary shaker incubator at 37 °C and 250 rpm. A 1% of the overnight culture were then grown at 37 °C in 3 mL M9 media with 1% glucose, 0.1% casamino acids, and tetracycline for about 4–6 hours and harvested when the absorbance at 600 nm reached 0.6–0.8. Cells were then resuspended in M9 growth medium with an OD 1.0. A drop of 50 μL of cells was dispensed onto the patterned PEI slide and incubated at room temperature for 30 min. To remove excessive cells, glass slides were washed gently using wash bottle in a circular motion without directly spraying onto the patterned area. Samples were then immediately inspected under the microscope.

Viability assay of the patterned cells:

To investigate the cell viability condition, a BacLight Viability Kit (BacLight, Invitrogen) was used by following manufacturer's protocol for staining the bacteria. The assay distinguishes live and dead bacterial cells by two colors: live bacteria cells present green fluorescence while dead bacterial cells present red fluroescence. A 2× BacLight solution was dispensed onto the slide and incubated at room temperature for 15 min in the dark. Samples were then rinsed with phosphate buffered saline (PBS) followed by Milli-Q water in a circular motion without touching the pattern and inspected under fluorescence microscope.

Fluorescence Microscopy:

Image acquisition was performed on a Nikon Eclipse TE2000-U inverted fluorescence microscope system equipped with fluorescence excitation and emission filters. Images were captured using a 1040×1392-pixel CCD camera (Photometrics Coolsnap ES, Roper Scientific Inc., Tucson, AZ), interfaced with the Metamorph imaging software (Molecular Devices, Sunnyvale, CA).

Atomic force microscopy:

AFM images were acquired in tapping mode with RTESP tips at a scan rate of 0.5 Hz on a Nanoscope III Dimension Atomic Force Microscope (Veeco Instruments Inc.) system. Thickness was quantified using a statistical analysis tool, Depth, of the Nanoscope software.