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Attachment of Motile Bacterial Cells to Prealigned Holed Microarrays

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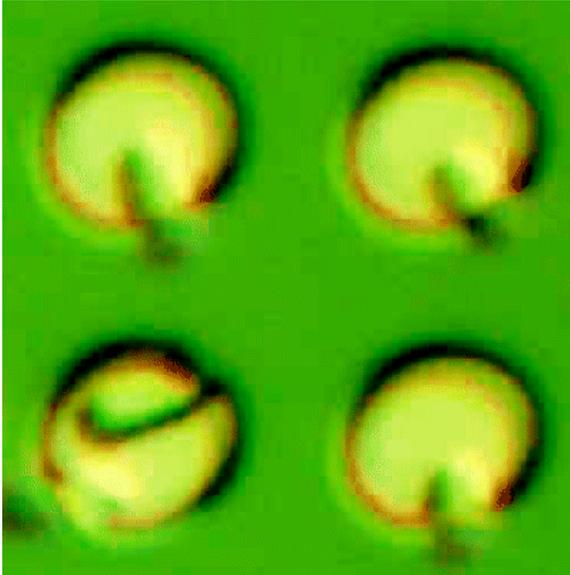
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SUBJECTS:

Bacteria, Microarrays, Molecular mechanics, Silicon, Surface structures

Abstract



Construction of biomotors is an exciting area of scientific research that holds great promise for the development of new technologies with broad potential applications in areas such as the energy industry and medicine. Herein, we demonstrate the fabrication of prealigned microarrays of motile *Escherichia coli* bacterial cells on SiO_x substrates. To prepare these arrays, holed surfaces with a gold layer on the bottom of the holes were utilized. The attachment of bacteria to the holes was achieved via nonspecific interactions using poly-L-lysine hydrobromide (PLL). Our data suggest that a single motile bacterial cell can be selectively attached to an individual hole on a surface and bacterial cell binding can be controlled by altering the pH, with the greatest occupancy occurring at pH 7.8. Cells attached to hole arrays remained motile for at least 4 h. These data indicate that holed surface structures provide a promising footprint for the attachment of motile bacterial cells to form high-density site-specific functional bacterial microarrays.

Introduction

Functional nanoelectromechanical systems (NEMS) with the ability to efficiently convert chemical energy to mechanical energy using simple fuels such as glucose or ATP at ambient temperature will open the door to hybrid nanodevices that can be assembled, maintained, and repaired using basic physiological and biochemical methods.¹⁻⁶ Bacterial cells may provide the perfect “power generators” for nanoscale biomotors since many bacterial cells are motile. Depending on the design of a biomotor and the food source, microorganisms may potentially power nanomachinery for extended periods of time. Initial calculations suggested that a biomotor based on motile bacterial cells can produce angular velocities of 0.05–0.2 rps assuming that bacterial cells are tethered to a surface in a random fashion.¹ This computational study suggested a 10% propulsive advantage if the minimum cellular separation distance is one-half the cell diameter.

Recently, predesigned motile bacterial cell microarrays were prepared by attaching *Escherichia coli* K-12 cells to micron-sized patterns prepared on a gold surface via microcontact printing.² On the basis of these data, a single motile *E. coli* bacterial cell can be attached to line or dot printed features with binding occurring primarily via the cell body. Surface-bound bacteria were shown to remain alive and motile after adhesion to a patterned surface for more than 4 h.² Thus, motile bacterial cells attached to a surface in the form of site-specific microarrays can

potentially be used to generate power for nanomachinery for extended periods of time. However, the attachment of motile bacterial cells to surfaces may also occur via their flagella, which has been suggested to markedly decrease biomotor efficiency and remains a major limitation to producing a viable proof-of-concept biomotor powered by motile bacterial cells.³⁻⁸ To overcome this problem, bacterial cells must be attached to a surface via their nose in a site-specific manner. In this way, all of the bacterial cells will have full use of their flagella, and all of the flagella will be oriented in the same direction, thus maximizing mechanical output. The importance of attaching bacterial cells to a surface in identical orientations is underscored by the examination of both external and internal forces of the system, which are operative on surface-attached motile bacterial cells. For bacteria attached to a surface in a “nose-on” orientation so that little or no flagella binding occurs and the bacterial cell is bound only through the cell body, the decrease in tumbling of bacterial cells alone will increase biomotor efficiency by ~20% because of hydrodynamic effects.¹ An additional efficiency increase is possible because of the absence of an artificial load placed on surface-adhered flagella.

To prepare microarrays of motile bacterial cells that may be suitable for a biomotor, we developed silicon substrates with holes etched by photolithography. The attachment of motile bacterial cells to these predesigned hole microarrays was achieved via nonspecific binding, which involves electrostatic interactions between oppositely charged molecular groups on the bacterial cell wall and the modified surface. Our data suggest that single motile bacterial cells can be selectively attached to individual holes on a surface and bacterial cell binding can be controlled by altering the pH.

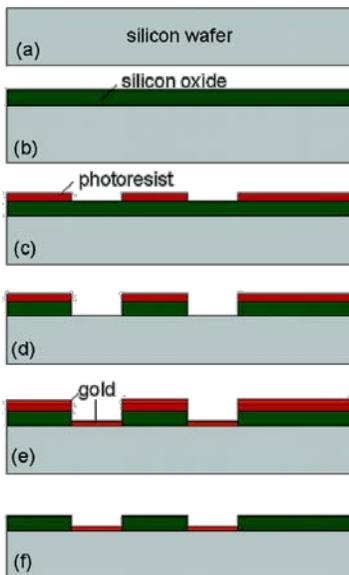
Materials and Methods

Materials.

Hydrogen peroxide (30%) and sulfuric acid were purchased from Fisher Scientific. *n*-Decydimethylchlorosilane was purchased from Gelest, Inc. (Morrisville, PA). 16-Mercaptohexadecanoic acid (MHA) and poly-L-lysine hydrobromide (PLL) (mol wt 70 000–150 000) were purchased from Aldrich/Sigma Chemical Co. (Milwaukee, WI). All chemicals were used as received without further purification. Milli-Q water (18.2 M Ω) was used for all aqueous experiments. Gram-rods of *E. coli* K12 were obtained from Carolina Biological Supply Company.

Substrate Preparation.

Photolithography was used to prepare hole arrays on SiO_x/Si wafers. Substrates with 3.0 × 0.5 μ m holes were prepared with a gold surface only at the bottom of the holes. Briefly, the fabrication of holed surfaces begins with a polished silicon wafer (Scheme 1, step a). An oxide layer is then grown on the wafer surface (Scheme 1, step b), followed by the deposition of a photoresist layer. The substrate is then patterned photolithographically at 0.5- μ m resolution (Scheme 1, step c). The photoresist layer is cured at 100 °C for 15 min after which it is used as a mask for wet chemical etching of the oxide using a buffered HF solution (Scheme 1, step d). A gold thin film is then deposited over the surface so the evaporated gold falls on the front surface as well as the bottom of holes. However, the vertical sidewalls of holes were not covered with gold since they were not in the line of sight of the incoming evaporated metal (Scheme 1, step e). The photoresist layer is then removed, lifting the top gold layer (Scheme 1, step f). The final device has a 10-nm gold layer on an oxide surface at the bottom of the hole and no metal on the sidewalls (Figure 1).



Scheme 1. Schematic for the Fabrication of Holed Surface Substrates^a

^a (A) Polished front surface of a silicon wafer. (B) An oxide layer is then grown on the wafer surface. (C) A layer of photoresist is then deposited using spin coating and patterned photolithographically with 0.5- μm resolution. (D) The photoresist layer is cured at 100 °C for 15 min after which it is used as a mask for wet chemical etching of the oxide using a buffered HF solution. (E) A gold thin film is deposited over the surface and the evaporated gold falls on the front surface as well as the bottom of holes leaving the vertical sidewalls silicon. (F) The photoresist layer is then removed.

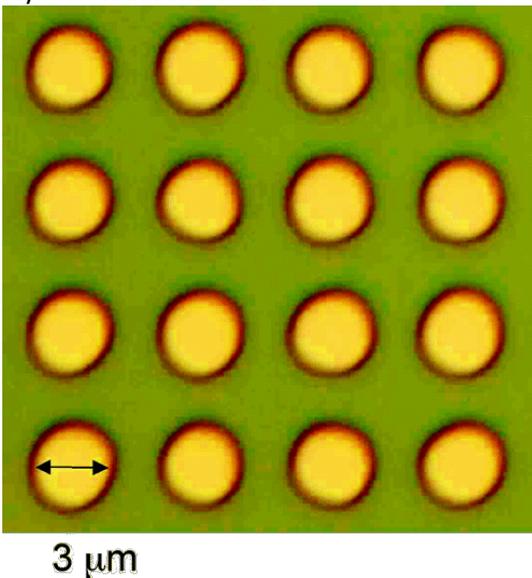


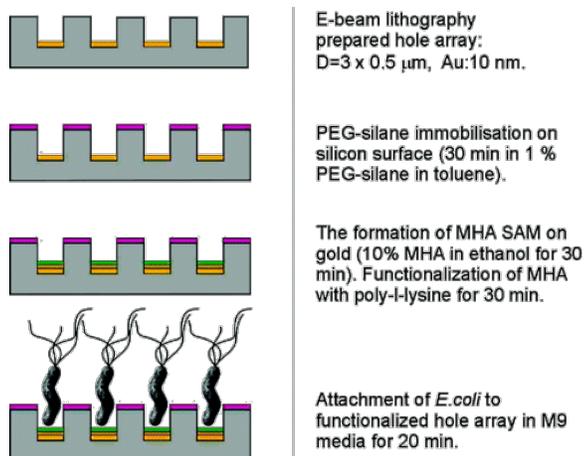
Figure 1 Optical image of a 3.0 \times 0.5 μm prefabricated holed surface.

Bacterial Cell Preparation.

E. coli K-12 cells were grown from a single colony in Luria-Bertani (LB) broth in a rotary shaker incubator at 37 °C and 225 rpm for 7–8 h. When the optical density (OD_{600}) of the culture reached ca. 0.8–1.0 (Agilent 8453 UV–vis spectroscopy system), the bacterial cells were centrifuged at 4000 rpm for 20 min and were resuspended in M9 media prepared from commercially available M9 minimal salts (Sigma, Milwaukee). The final bacterial cell concentration was approximately $(1\text{--}2) \times 10^7$ cells/mL, which was determined by measuring the absorbance at 600 nm and by cellular counting methods using a Bright-Line hemacytometer (Fisher Scientific).

Microarray Preparation.

Bacterial attachment to prefabricated holed surface arrays was accomplished by treating the sample surface as indicated in Scheme 2. Initially, the substrates were washed in piranha solution at 40 °C for 5 min to remove any form of organic contaminant from both the gold and silicon surfaces. To prevent nonspecific adsorption of bacterial cells to silicon surfaces, the substrate was passivated with *n*-decyldimethylchlorosilane from a 1% solution in toluene for 30 min. The substrate was then immersed into a 10 mM solution of MHA in ethanol for 30 min providing a self-assembled monolayer (SAM) of MHA on the gold-coated surfaces of the hole bottoms. MHA coated regions were then functionalized using an aqueous solution of PLL. Finally, the modified substrates were immersed in M9 media containing $(1-2) \times 10^7$ cells/mL *E. coli* K-12 bacterial cells for ~20 min at 37 °C, after which the substrate was rinsed with fresh M9 solution and Milli-Q water.



Scheme 2. Schematic for the Steps Used to Fabricate a Bacterial Array on Prefabricated Hole Surface Structures^a (1) Washing substrates in piranha solution at 40 °C for 5 min, (2) *n*-decyldimethylchlorosilane immobilization of the silicon surface for 30 min in 1% solution in toluene, (3) self-assembly of MHA molecules on gold layers from 10% MHA solution in ethanol for 30 min; functionalization of MHA with poly-l-lysine for 30 min from PLL aqueous solution. (4) Attachment of *E. coli* to MHA-PLL functionalized hole array in M9 media for 20 min.

Imaging.

Fabricated microarrays were characterized by optical and atomic force microscopy (AFM). Either a Veeco CP-Research or Nanoscope IV (Nanoman) was employed to acquire topography and frictional force images. The cantilever tip (Model # MSCT-AUHW, purchased from Veeco) had a spring constant of 0.05 N/m. Optical images were obtained with an Axiovert 100A optical microscope equipped with a Penguin 600CL digital camera and StreamPix software.

Results and Discussion

The addition of motile *E. coli* bacterial cells to prefabricated hole array samples at pH 6.8 resulted in *E. coli* attachment to the MHA-PLL modified gold-coated holes (Figure 2). Optical monitoring *E. coli* K12 bacterial attachments indicate that binding occurred in ~90% of the holes under these experimental conditions and not to the *n*-decyldimethylchlorosilane passivated SiO_x surfaces (Figure 2). The motility of adsorbed bacterial cells was monitored at room temperature with an optical microscope using a liquid cell containing the substrate immersed in fresh M9 media (Movie S1, Supporting Information). On the basis of direct optical and fluorescent monitoring, surface-adhered *E. coli* cells remained alive and motile for a minimum of 4 h (Figure S1, Supporting Information).⁹ To further characterize bacterial cell binding to the holed surface arrays, samples containing *E. coli* cells were dried in air, and AFM studies were conducted. On the basis of AFM/LFM images, *E. coli* cells only

adhere to the patterned areas at the bottom of the holes and not to the passivated portions of the substrate (Figure 3 A–C). Therefore, PLL-modified surfaces, as prepared herein, appear to be excellent for the attachment of motile *E. coli* bacterial cells, and *n*-decyldimethylchlorosilane significantly inhibits cell adhesion.

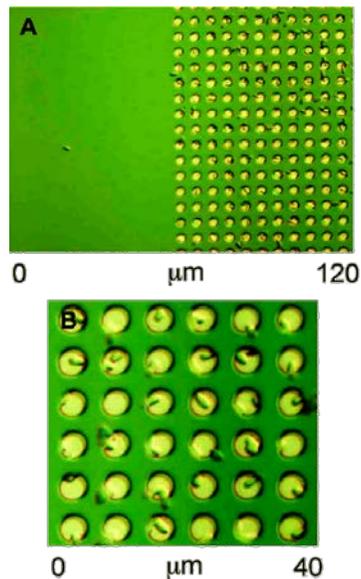


Figure 2 Optical images of *E. coli* attachment to a $3.0 \times 0.5 \mu\text{m}$ prefabricated holed surface array at pH 6.8 demonstrating (A) excellent resistive and (B) link properties of the used chemicals.

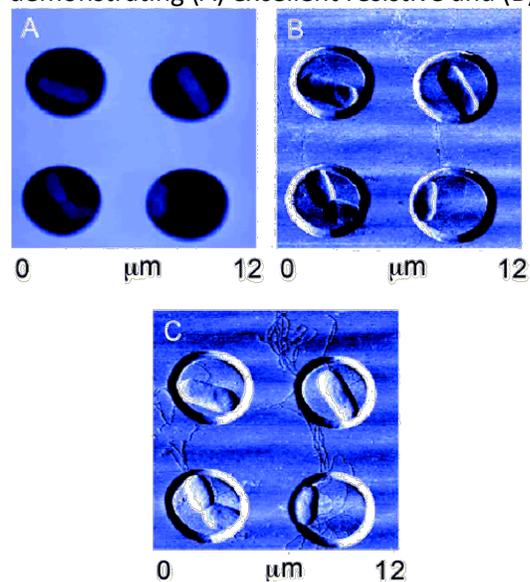


Figure 3 (A) AFM and (B and C) LFM images of dried *E. coli* cells bound to a $3.0 \times 0.5 \mu\text{m}$ prefabricated holed surface array at pH 6.8.

In an effort to examine the mode of binding of *E. coli* K-12 cells to the prefabricated holed surface structures, we examined *E. coli* binding as a function of pH (Figure 4). Six PLL solutions at different pH values (4.7, 5.6, 6.6, 7.5, 8.7, and 9.6) were prepared by adding the appropriate amount of acetic acid or sodium hydroxide to the original PLL solution (pH 6.8). Each substrate was soaked in a PLL solution at different pH values for ~ 10 min followed by extensive washing with Nanopure water. The MHA-PLL modified holed surface substrates were then immersed in M9 media containing $(1-2) \times 10^7$ cells/mL of *E. coli* for ~ 20 min in an incubator at 37°C . Finally, the substrates were washed with Nanopure water for 2 min. As shown in Figure 4, bacterial attachment increased significantly as the pH was increased from 4.7 to 9.6. In the pH range studied, MHA and PLL remain oppositely charged since

their pK_a values are 5 and 10.5, respectively, Scheme 3. Raising the pH of the PLL solution results in an increase in the degree of deprotonation of MHA, thereby providing a stronger electrostatic interaction between MHA and PLL molecules. The MHA-PLL assemblies remain stable during bacterial attachment from M9 media since the pH of M9 media, as prepared, was 6.8 resulting in no decomposition of the MHA-PLL structure. The best conditions for bacterial attachment to prefabricated holed surface structures with little or no nonspecific binding to the silicon oxide surface were observed at pH 7.8 (Figure 4). At higher pH values, increased nonspecific binding of *E. coli* K12 bacteria to the silicon surface was observed suggesting that PLL may bind to the negatively charged silicon oxide surface. To test this hypothesis, PLL patterns were generated on a silicon surface via microcontact printing using a PDMS stamp coated with a basic (pH 9.0) PLL solution. Upon the addition of *E. coli* K-12 bacterial cells, cellular attachment was observed only to the PLL modified portion of the silicon oxide surface (Figure 5), consistent with the conclusion that the observed adventitious binding to the prefabricated holed surface structures at higher pH values is due to PLL binding to the silicon surface. Therefore, the observed increase in attachment efficiency of *E. coli* K-12 bacteria to MHA-PLL-modified gold is likely due to electrostatic interactions between the negatively charged *E. coli* cells⁹ and the protonated amino groups (NH_3^+) of poly-L-lysine.¹⁰

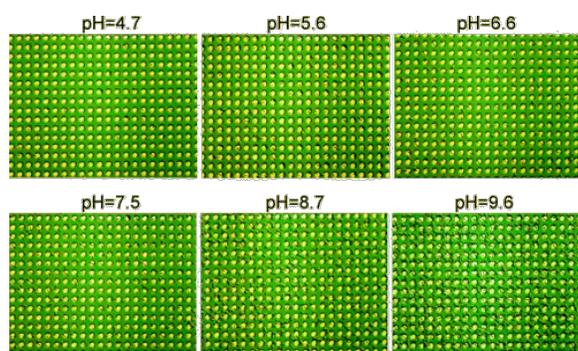
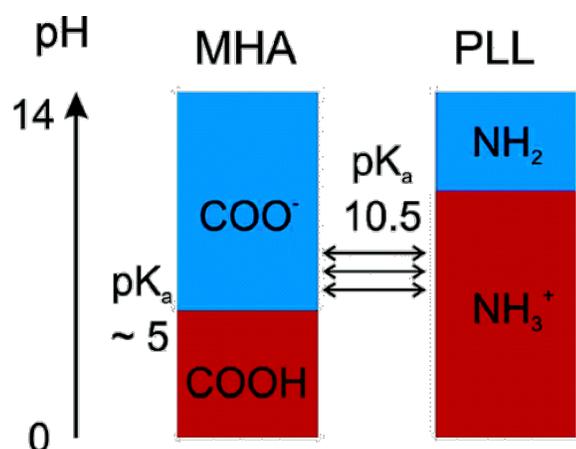


Figure 4 Optical images of *E. coli* attachments to $3.0 \times 0.5 \mu\text{m}$ prefabricated holed surface arrays demonstrating the pH dependence of electrostatic interaction between the cells and MHA-PLL-modified gold surface within pH range of 4.7–9.6.



Scheme 3. Schematic of the pH Dependence of MHA and PLL Providing the Expected Electrostatic Interactions between PLL and Bacterial Cells

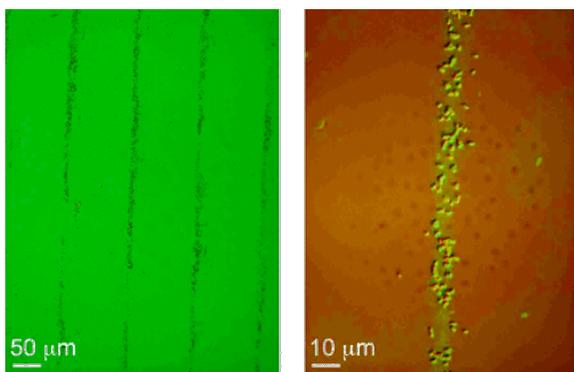


Figure 5 Optical images of *E. coli* K12 bacterial cell attachment to PDMS stamped PLL lines on a bare silicon oxide surface at a pH of 9.1.

In conclusion, motile *E. coli* K-12 bacterial cells can be attached to chemically modified, prefabricated holed surface structures via an electrostatic interaction between negatively charged groups of the bacterial cell surface with positively charged PLL assemblies. The attachment *E. coli* K-12 bacterial cells were controlled by varying the pH of the PLL solution and were significantly improved when the pH of the PLL solution was basic (pH 7.8). These data indicate that prefabricated holed surface structures provide a promising footprint for the attachment of motile bacterial cells.

Supporting Information Available

A movie of surface-attached motile bacterial (MovieS1.mov) and a figure (Figure S1) showing the fluorescent study for cellular viability. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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