Published in final edited form as:

Langmuir. 2008 April 15; 24(8): 4161–4167. doi:10.1021/la7038653.

Efficient Immobilization and Patterning of Live Bacterial Cells

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Abstract

A monolayer of live bacterial cells has been patterned onto substrates through the interaction between CFA/I fimbriae and the corresponding antibody. Patterns of live bacteria have been prepared with cellular resolution on silicon and gold substrates for *Salmonella enterica* serovar Typhimurium as a model with high specificity and efficiency. The immobilized cells are capable of dividing in growth medium to form a self-sustaining bacterial monolayer on the patterned areas. Interestingly, the immobilized cells can alter their orientation on the substrate, from lying-down to standing-up, as a response to the cell density increase during incubation. This method was successfully used to sort a targeted bacterial species from a mixed culture within 2 h.

Introduction

Bacterial cells are ideal sensors for environmental monitoring because of their low cost, fast growth, rich genetic modifications, easy handling, and sensitivity to a wide variety of environmental stimuli.¹ Efficient, controllable immobilization of bacteria is critical for the success of such biosensors. Such immobilization also offers potential applications in biomedical research and fundamental bacteriological studies such as quorum sensing. The majority of reported immobilization approaches utilize either nonspecific adsorption of bacterial cells on chemically treated surfaces or physical entrapment of cells in gels or microholes. For example, attachment of bacteria has been conducted on prefabricated microarrays with microholes treated either with poly-L-lysine (PLL)² or with *n*-hexadecanethiol,³ while the areas surrounding these holes were coated with a layer of poly(ethylene glycol) (PEG). Micro-⁴ and macrocontact⁵ printing have been employed to transfer live bacteria onto the surface of a nutrient-containing matrix such as agarose or hydrogel. Bacterial microarrays have also been prepared by loading individual bacterial cells into microwells (2.5 μm wide, $\sim 3 \mu\text{m}$ deep) at the distal end of an optical fiber bundle by centrifugation.⁶ A bacterial array printed onto porous nylon has also been reported,⁷ in which cells were physically entrapped in the pores of a special nylon substrate in close contact with a nutrient medium. Such arrays offer great potential for monitoring genotoxicity⁸ and heavy metals in the environment⁹ and for high throughput assays of gene expression.¹⁰ Very recently, Akselrod et al. reported three-dimensional heterotypic arrays of living cells in hydrogels created by means of high-precision (submicrometer accuracy) time-multiplexed holographic laser trapping.¹¹ However, this technique has limited applications in practice, in that, besides

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Supporting Information Available: Images of immobilized *S. Typhimurium* cells and the flagella expressed by the immobilized cells (Figures S-1 and S-2), and movie clips (Movies S-1 and S-2) showing the motion and dividing of immobilized cells. This material is available free of charge via the Internet at <http://pubs.acs.org>.

the need for a trapping laser, excessive exposure to laser light may cause photodamage to the cells; furthermore, arrays are expected to merge in a few hours because of cell division.

Another approach to bacterial immobilization takes advantage of the interaction between an appropriate antibody–antigen pair. This approach has been used in conjunction with microcontact printing and dip-pen nanolithography;¹² binding of *Escherichia coli* to microscale features was achieved through antibodies against the whole cell or bacterial flagella. However, *E. coli* cells showed a lower attachment to features modified with antibodies than with PLL.¹² The poor immobilization of bacterial cells mediated by antibody binding is also evidenced by a report on environmental toxicity monitoring using immobilized *E. coli* in which only 2% surface coverage of the bacteria was achieved.¹³ In other studies, antibody-modified substrates have been used for immobilizing and detecting pathogenic bacteria, but little has been reported on the cell density on the substrates.¹⁴ The present study, as well as previous studies, indicates that antibody–antigen-based immobilization does not hinder such physiological activities as cell division (this work), gene expression, or bioluminescence of bacteria at the locations of their immobilization.^{12,13}

In most of these applications, limited attention has been paid to the efficiency and control of the immobilization of *live* cells on substrates or to the physiological activity of individually immobilized bacteria. For example, patches of bacteria created by microcontact printing will either grow in lateral directions or disintegrate in a short period of time if exposed to a flow reactor, resulting in a loss of functionality of the sensor. An efficient, reproducible, stable, self-sustaining, and highly specific immobilization of bacteria on a predefined surface is necessary. In this paper, we report such an immobilization of live cells of *S. Typhimurium* on well-characterized material surfaces; we chose this species because of its zoonotic properties, infecting both animals and humans, and our desire to prevent such infections.

Experimental Section

Bacteria

In most experiments, *Salmonella enterica* serovar Typhimurium $\Delta asd::kan^R$ H71-pHC was used as a model bacterial species for immobilization and patterning. We constructed a $\Delta asd::kan^R$ lethal mutant from wild type *S. Typhimurium* H71, termed $\Delta asd::kan^R$ *S. Typhimurium* H71.¹⁵ This lethal mutant cannot normally survive unless an *asd* gene is presented *in trans*. Plasmid pHC, which contains a chimeric triple promoter, *PtetA~PpagC~PphoP*,^{16,17} was used to express CFA/I fimbriae. The chimeric triple promoter was installed upstream of the *cfa/I* operon to enhance *cfa/I* expression. For the sorting experiment (Figure 6), *S. Typhimurium* $\Delta asd::kan^R$ H71-(pHC+pGFP) and *E. coli* O157:H7 RFP were used. Plasmids pHC and pGFP (Clontech, Mountain View, CA) were used to express CFA/I fimbriae and green fluorescence protein (GFP), respectively. *E. coli* O157:H7 RFP expressing red fluorescence protein (RFP)¹⁸ was obtained from Dr. T. Khan and Dr. B. Klayman at the Center for Biofilm Engineering, Montana State University.

Frozen bacteria stock at $-80\text{ }^{\circ}\text{C}$ was inoculated onto a Luria–Bertani (LB) plate and incubated at $37\text{ }^{\circ}\text{C}$ overnight. The bacteria were then inoculated into an LB liquid medium without antibiotics and shaken at 125 rpm at $37\text{ }^{\circ}\text{C}$. The bacterial cells were harvested when the optical density of the medium at 600 nm (OD600) reached about 0.5–0.6, which corresponds to a colony forming unit (CFU) value of $\sim 9.0 \times 10^8/\text{mL}$.

Antibody

The anti-CFA/I serum was prepared by immunizing a rabbit intramuscularly (im) with purified CFA/I fimbriae proteins. Four weeks post immunization, the rabbit was bled to check the serum anti-CFA/I titers using an enzyme-linked immunoadsorbent assay (ELISA). Serum IgG was

further purified with the protein G column to remove the nonspecific serum protein. This antibody was diluted to 100 times with phosphate-buffered saline (PBS) (pH = 7.4) before use.

Chemicals

PBS buffer salt, 3-aminopropyltriethoxysilane (APTES), and 11-mercaptopundecanoic acid (11-MUDA) were purchased from Sigma-Aldrich (St. Louis, MO). *N*-[β -Maleimido-propyloxy]-succinimide ester (BMPS), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (NHS) were purchased from Pierce Biotechnology (Rockford, IL). Bacterial viability dyes (Live/Dead *BacLight*) were purchased from Invitrogen (Carlsbad, CA). HS(CH₂)₁₁(OCH₂CH₂)₃-OH (SPT-11) was purchased from Sensopath Technology, Inc. (Bozeman, MT), and 2-[methoxy(polyethyleneoxy)-propyl]trimethoxysilane (PEG-silane) was purchased from Gelest, Inc. (Morrisville, PA).

Substrate Passivation

Gold—Gold-coated silicon chips were cleaned in a chloroform bath and ozone plasma chamber, rinsed with 100% ethanol, and then incubated in a 2 mM solution of SPT-11¹⁹ in 100% ethanol overnight. After being rinsed with copious (5 × 1 mL) ethanol and water, the modified chips were dried with nitrogen.

Silicon—Silicon chips cleaned by ozone plasma were heated in a solution of PEG-silane²⁰ (2%, v/v) in isopropanol at 60 °C for 70 min, rinsed with copious (5 × 1 mL) 100% ethanol, and dried with nitrogen.

Patterning Substrates Using a Microfocused Ga⁺ Ion Beam

The substrate was patterned using a Ga⁺ beam of a time-of-flight secondary ion mass spectrometry (ToFSIMS) system (TRIFT I, PHI-Evans, Chanhassen, MN).²¹ The microfocused Ga⁺ beam provides a 1.3 nA DC current at ~15 keV beam energy. The sample potential for positive ions was kept at ~3 kV; hence, the primary ion impact energy was ~12 keV. The direct Ga⁺ ion current at the target position was measured to be ~1.26 nA. The etching time was carefully adjusted so that only a very thin layer of the substrate surface was removed (<5 nm). To the best of our knowledge this is the first report on the preparation of bacterial cell patterns using a focused ion beam.

Covalent Linking of Antibody

Gold—Etched gold substrates were incubated in a 2 mM solution of 11-MUDA in 100% ethanol overnight. After being rinsed with copious (5 × 1 mL) ethanol and water, the modified chips were treated with a NHS/EDC solution (NHS, 3 mg/mL; EDC, 2 mg/mL) for 1 h at ambient temperature and further rinsed with PBS buffer.

Silicon—Etched silicon substrates were incubated in a solution of APTES in methanol (2%) for 10 min and rinsed with copious (5 × 1 mL) ethanol before being further incubated in a solution of BMPS in anhydrous acetonitrile (10 mM) for 30 min at room temperature. The chips were rinsed with acetonitrile and dried in air.

Antibody Linking—The activated substrates were incubated with antibody solution for 1 h at room temperature and rinsed with PBS buffer to remove the free antibody molecules. These antibody-modified chips were then used for bacterial immobilization.

Immobilization of Bacterial Cells

Substrates with antibody patterns were incubated with a suspension of live bacterial cells in half LB growth medium (LB medium diluted with 1% NaCl solution by a ratio of 1:1, v/v) at

room temperature for 3 h. Some samples were also incubated at 37 °C for 3 h and showed no obvious difference. After incubation, the samples were gently rinsed with copious PBS buffer (>5 mL) to remove the planktonic or loosely attached cells. The rinsed samples were kept in PBS buffer or growth medium at 4°C for further analysis.

Optical Imaging of Immobilized Cells

Optical imaging was done using either an Olympus BX61 or a Leica TCS SP2 microscope. All of the images were recorded in reflection mode (Figures 2, 4, and 5A,B) or in fluorescence mode (Figures 5C,D and 6). For reflection mode imaging, the samples were imaged using water immersion objective lenses in PBS buffer or 1/2 LB growth medium without staining the bacterial cells. The bright background color of the patterned areas in Figures 2, 4, and 5A,B is due to the Ga⁺ focused ion beam etching of the substrate surfaces. For fluorescence mode imaging, the cells were stained using Live/Dead *BacLight* according to the protocol suggested by the dye manufacturer, and imaged in a PBS buffer using water immersion objective lenses.

X-ray Photoelectron Spectroscopy (XPS)

XPS analysis was conducted using a Physical Electronics 5600ci system equipped with monochromatized Al K α X-rays. Data acquisition and data analysis were performed using RBD AugerScan2 software.

Atomic Force Microscopy (AFM)

Images were obtained in tapping mode in air using a Multimode V atomic force microscope from Veeco (Santa Barbara, CA) with an E- or J-type scanner and using NSC18 AFM probes from MikroMasch (Wilsonville, OR).

Field Emission Scanning Electron Microscopy (FESEM)

Immobilized and planktonic bacteria deposited on a clean silicon wafer were imaged using a Zeiss SUPRA 55VP system (Carl Zeiss, Germany).

Results and Discussion

Figure 1 shows the method for immobilizing and preparing microscale patterns of live bacterial cells. Depending on the choice of substrate, a clean silicon wafer or a gold substrate surface is first passivated using a PEG layer to prevent nonspecific adsorption of the antibody.^{19,22} The substrate is then patterned using a focused Ga⁺ ion beam to remove the preselected portions of the passive layer from the substrate. This is followed by attaching one end of a cross-linker molecule to the sputtered area of the substrate and the other end to an antibody molecule, all via covalent bonds. For silicon, trialkoxysilanes are used to couple the cross-linkers and PEG moieties to the substrate, and for gold substrates thiol-based compounds are used. After rinsing with PBS buffer to remove the excess antibody from the medium, the antibody-patterned substrate is incubated with a bacterial suspension at ambient conditions for a period of about 3 h. The bacterial cells attach only to the antibody-modified areas, so that a micropattern of bacterial cells is achieved.

Optical images of bacterial patterns on gold and silicon substrates obtained while the bacteria were alive are shown in Figure 2. A sharp contrast between the patterned area and the PEG-passivated area is clearly demonstrated in these images. Only sparsely attached cells are observed in the PEG-passivated area, and all of the patterned area is occupied by a dense monolayer of *S. Typhimurium* cells. To obtain such high-quality micro-patterns of bacterial cells, attention must be paid to surface preparation and substrate cleanliness during the passivation step (Figure 3). Flaws in surface passivation can cause failures of patterning. The

failure of passivation facilitates antibody adsorption everywhere on the surface, leading to indiscriminate immobilization (Figure S-1 in the Supporting Information).

The attachment of antibody-immobilized cells was robust enough to resist washing with copious buffer solution for all strains tested. In some experiments, the antibody molecules were allowed to adsorb noncovalently onto the patterned (unpassivated) areas without the cross-linker. Bacterial patterns generated in this way were still resistant to the wash with PBS buffer (though not as strong as covalently coupled antibodies), indicating that the attachment, while not as strong, was robust enough for bacterial immobilization. This is important in practice and avoids the extra step of coupling the antibodies covalently to the substrates. Figure 2A is an example of such immobilization.

The immobilized cells remain viable for at least 6 h in PBS buffer when stored at 4 °C, as evidenced by the fluorescence of the immobilized cells after being stained with viability dyes (Figure 5C). The viability of the immobilized cells in PBS buffer is also supported by their free motion around the locations of immobilization, as shown in Movie S-1 in the Supporting Information. In growth medium, the immobilized cells can last for at least 3 days while retaining their physiological activities, and a longer lifetime can reasonably be expected for the immobilized cells, provided nutrients are available and the antibody molecules remain active. Monitoring the immobilized bacteria under optical microscope showed clearly that the cells were alive and capable of division in the growth medium at ambient conditions (Figure 4 and Movie S-2 in the Supporting Information). It is clear that the bacteria were dividing without hindrance even though they were tied to the substrate through antibody–antigen interactions.

As seen in Figure 2D, cellular resolution of bacterial patterning was achieved in our experiments. Some of the line thicknesses, $\sim 1\ \mu\text{m}$, in the patterns are comparable to the dimensions of the bacteria, and the bacteria are concentrated along these narrow lines while very few cells are observed outside the lines. In fact, those few cells immobilized outside the patterned areas are also most likely immobilized through antibody–antigen interactions. Passivation is not perfect: some antibodies find their way to the substrate on the defects of the passive layer. These antibodies outside the patterned areas become centers of attachment for the motile bacteria that happen to be at these locations.

Poly-L-lysine has been successfully used to immobilize *E. coli* K-12 strains on abiotic substrates^{2,12} as well as to attach eukaryotic cells to glass slides.^{23,24} However, it failed to immobilize all the bacterial species we tested, including *E. coli*, *S. Typhimurium*, and *Haemophilus influenzae*. Other approaches to immobilizing bacterial cells, such as an amino-terminated surface,²⁵ a positively charged polymer,²⁶ a gelatin-coated surface,²⁷ and a direct linking of bacterial cells via covalent bonds,²⁸ proved to be unreliable, inefficient, and irreproducible for the strains we tested. The difficulties associated with these techniques can partially be explained by the fact that bacterial cells have a very small contact area with the substrate surface due to their smaller size relative to eukaryotic cells, typically by 1–2 magnitude orders. Furthermore, a forest of appendages, such as pili and flagella, protruding out of the surface, as shown in the inset of Figure 2A, prevent bacteria from contacting the surface. Additionally, many bacterial species, including *S. Typhimurium*, have a layer of extracellular polymeric substances surrounding the bacteria (capsular EPS)²⁹ which prevents the bacteria from direct contact with the surface.

The choice of antibody is critical to the success of bacterial immobilization. The purity of the antibody is crucial; an affinity-purified antibody is preferred, because the serum contains a considerable amount of proteins other than the desired antibody, for example, serum albumin, which will compete for the bioactive cross-linker sites on the surface during the covalent linking of antibody proteins to the substrate. This will then mostly coat the surface with serum albumin,

reducing the fraction of the surface covered with antibody molecules. Therefore, the use of a serum without affinity purification will yield a low density of antibody molecules on the substrate and thus give rise to a low density of immobilized bacteria. This assumption is supported by our early attempts to immobilize bacterial cells through unpurified anti-CFA/I serum, which only afforded unreliable poor immobilization. The efficiency of bacterial immobilization also depends on the choice of antibody–antigen pair. Bacterial species express a large number of surface antigens which play various roles in bacterial virulence and adhesion.³⁰ Antibodies against many of these surface antigens are commercially available, but we recommend that those appendages that protrude outside the cell walls and do not have rapid movements independent of the bacteria be considered. For example, anti-flagellin is not a good choice for leashing bacteria to substrates because flagella, as the bacterial motility organ, rotate with very high speeds, exceeding 10 000 rpm at 35 °C,³¹ vastly reducing the chance of antibody–antigen interactions. The interaction between an antibody–antigen pair is fairly weak: a force of ~50 pN is required to break the antibody–antigen interaction,³² which is more than 1 order of magnitude smaller than a covalent bond (>1 nN). At this time, we do not know how much force a flagellum imparts to a *S. Typhimurium* cell, but, to immobilize a bacterium, we hypothesize at least one antibody–antigen interaction is required to hold the bacterium in place. For the reasons discussed above, in our experiments, the CFA/I fimbriae were chosen as the target antigen because (1) they are long and protrude outside the cell body (inset of Figure 2A), (2) the fimbriae are expressed in *S. Typhimurium* strains in abundance, and (3) these appendages do not rotate rapidly independently of the bacteria as do the flagella. A typical *S. Typhimurium* cell will fit into a $2 \times 1 \mu\text{m}^2$ area (inset Figure 2A), which predicts a maximum packing density of about one bacterium per $2 \mu\text{m}^2$. This value is in good agreement with our observations conducted under optical microscope (Figure 2A,C and the Supporting Information).

The unique features of the bacterial cells patterned in this way are the stability and self-sustaining capability of the patterns. As shown in Figure 4 and Movie S-2 in the Supporting Information, the immobilized cells are capable of dividing and regenerating. The daughter cells either stay attached to the antibody-modified area or enter the growth medium after the antibody-modified area is fully occupied. Therefore, the patterns are always covered by a monolayer of live cells and do not change physical dimensions as a result of cell division. In a flow reactor, cells released into the liquid phase will be carried away and the self-refreshing bacterial pattern can last as long as the antibody remains active, provided that the medium has the nutrients to sustain life. This is particularly useful for those applications, such as water quality control, that require continuous monitoring of pollutants as an environmental sensor. These features are not available for patterns prepared by mechanically transferred cells on an agar plate;^{4,5} for sensors fabricated by incorporating cells in alginate,³³ agar,³⁴ or sol–gel matrices;³⁵ or for arrays constructed by physical entrapment into microwells.⁸ For these patterns, bacteria are held on the substrates or inside the microwells with weak forces, and the cells do not stay fixed to the patterned areas for long periods of time. These patterns will eventually degrade and disintegrate when exposed to a liquid medium or a flow reactor.

The observation of patterned bacteria on antibody-modified substrates allows probing of the individual or collective behavior of the bacteria. For example, we can follow the increase in surface population density of bacteria incubated in a growth medium. Such observation in our experiments yielded a surprising result, as shown in Figure 5A,B, in which bacteria immobilized in a lying-down orientation took a standing-up orientation as their density increased. This standing-up orientation of the crowded cells was also confirmed by laser scanning confocal microscopy (LSCM) images (Figure 5C,D). Although this behavior is not well understood at this time, it might be related to the depletion of nutrients at the crowded bacterial positions and to the struggle of the bacteria to move away from their immobilized positions. This hypothesis is supported by the observation of an excess number of flagella

produced by the immobilized bacteria (Supporting Information Figure S-2), presumably in an effort to free themselves from their positions.

The use of purified antibodies targets specific antibody–antigen interactions; hence, only bacteria with the targeted antigens are immobilized. This specificity of immobilization can be used in many applications, including sorting organisms from a mixed culture. A successful sorting of *S. Typhimurium* (green) from a culture mixed with *E. coli* (red) is demonstrated in Figure 6. The mixture (Figure 6A) was allowed to interact at ambient conditions for only 2 h with a substrate patterned in a checkerboard geometry with antibodies specific to *S. Typhimurium*. Figure 6B shows only the desired organism (*S. Typhimurium*) immobilized on the antibody patterns: *E. coli* cells were washed off the substrate. Sorting a specific bacterial strain typically takes weeks to months, because the mixed culture is subjected to the repeated inoculation and growth of sequentially diluted cultures on preselected agar plates.³⁶

Could bacteria be linked directly to a substrate without a need for an antibody? The advantage of this method, if successful, would be to avoid the necessity of finding an antibody for a given bacterial strain. The disadvantage would be that all the proteins, and possibly other biomolecules, in the medium would have a chance at immobilization; hence, the bacterial specificity would be lost. We tested this idea by linking bacterial cells directly to a substrate through covalent coupling between bacterial membrane proteins and cross-linkers on the substrate, as was done with the antibodies. The activated substrates, with either carboxyl groups or maleimido groups, were directly incubated with a suspension of bacterial cells in the growth medium and also in the PBS buffer solution for ~15 h under ambient conditions. The results (not shown) indicate only sparsely attached cells populating the surface. We hypothesize the reason for the failure of direct immobilization is that the activated surface was blocked by proteins in the bacterial suspension before the bacterial cells could reach the surface. These proteins are components of the growth medium or are secreted by the bacteria in the suspension. It is difficult to remove these proteins completely, even by washing the cells with PBS buffer repeatedly. Furthermore, the capsular EPS layer surrounding each bacterium would prevent the bacterial surface proteins from making direct contact with the activated substrate surface.

Summary

Our results demonstrate that cellular resolution can be achieved for the preparation of self-renewing bacterial patterns on abiotic substrates through antibody–antigen binding with high efficiency and controllability. The efficiency of the immobilization is attributed to the careful selection and assembly of the substrate surface chemistry at each step and to the affinity-purified antibody raised specifically against a carefully targeted antigen protruding outside the cell surface of the bacteria, the CFA/I fimbriae of *S. Typhimurium*. Patterned cells remain viable under this *immobilized* condition and are capable of reproducing. The technique is readily applicable to sorting specific bacteria from a mixed culture within hours as compared to the standard bacteria purification methods, which typically take days.

Supplemental Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work is funded by NASA-EPSCOR under Grant NCC5-579, by MSU CBIN, by U.S. Public Service Grant AI-41123, and by Montana Agricultural Station and USDA Formula funds. We thank Mr. M. Deliorman for his help on TOFSIMS and Dr. B. Pitts for her help on optical microscope imaging.

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Figure 1.

Micropatterning of live bacterial cells. The substrate was first modified with chemicals that inhibit the nonspecific adsorption of proteins (blue bars) and then etched using a programmable focused Ga^+ ion beam. The freshly etched surface was then modified with a cross-linker (orange bars) to link the antibody (cyan Y shapes), raised against the bacterial surface antigens, to the substrate. When the patterned substrate was incubated with the bacterial suspension, the bacterial cells adhered only to the antibody-modified area and thus formed a monolayer of bacterial cell patterns.

Figure 2.

Micropatterns of live *S. Typhimurium* cells immobilized on substrates etched by a focused ion beam. (A) Square pattern on gold. The inset in (A) shows a high-resolution atomic force microscope image of a typical *S. Typhimurium* $\Delta asd::kan^R$ H71-pHC, obtained in air, revealing the CFA/I fimbriae (scale bar: 1 μm). (B) Line pattern on silicon. (C) Bobcat mascot and MSU-ICAL logo on silicon. (D) Enlargement of the area within the white dotted box in (C), demonstrating the cellular resolution of cell patterning.

Figure 3.

Characterization of silicon and gold substrates. (A) XPS spectra of silicon chips (i) modified with APTES; (ii) modified with APTES and BMPS; and (iii) modified with antibody. No N1s signal was observed for unmodified silicon, and both C1s and N1s signals increased as APTES, BMPS, and antibody were linked to the silicon surface (from i to iii), implying that the antibody was successfully bonded to the silicon surface with APTES/BMPS as the cross-linkers. (B) XPS spectra of silicon chips (i) cleaned by O₃ plasma; (ii) passivated using PEG-silane; and (iii) passivated and incubated with anti-CFA/I antibody. The increase of the C1s peak from (i) to (ii) suggests the success of the passivation of the silicon surface using PEG-silane, while the absence of the N1s peak in (iii) confirms the resistance against antibody adsorption of the passivated silicon surface. (C) XPS spectra of gold substrates (i) modified with 11-MUDA and (ii) with anti-CFA/I antibody cross-linked. For the gold surface, C1s, N1s, and O1s signals were used to monitor the substrate modification. The prominent N1s signal in (ii) and the increase of O1s from (i) to (ii) indicate the attachment of the antibody. (D) XPS spectra of gold substrates (i) cleaned by O₃ plasma; (ii) passivated with SPT-11; and (iii) passivated and incubated with anti-CFA/I antibody. The increases in the C1s and O1s peaks from (i) to (ii) suggest the success of the passivation of the gold surface using SPT-11, while the absence of the N1s peak in (iii) confirms the resistance against antibody adsorption of the passivated gold

surface. (E) AFM image of antibody molecules covalently linked to the silicon surface. Antibody molecules linked to the gold surface were not imaged by AFM because of the interference from the gold nanograins.

Figure 4.

(A) Selected time lapse images over an incubation period of 270 min, showing the regeneration of immobilized *S. Typhimurium* on a patterned silicon substrate that was incubated in growth medium at room temperature. The red ovals highlight the dividing cells. (B) Time lapse image at 270 min showing that newly divided cells only occupy the available patterned areas and that excess cells are released into the growth medium and can be removed by gently rinsing.

Figure 5.

Immobilized *S. Typhimurium* cells change their orientations according to the surface population density on the substrate. Images (A) and (B) correspond to a sample incubated in growth medium at 37 °C for 3 and 15 h, respectively. Notice that while the cells are lying down in part (A), they appear to stand up in (B), when the surface is crowded (see Figure 5D). LSCM image of a sample incubated in growth medium for 15 h and then stored in PBS buffer at 4 °C for 6 h and stained with viability stains (C) indicating that the majority of the cells were alive. The reconstituted Z-section image of the immobilized cells (D) further confirms that a majority of the cells took a standing-up orientation.

Figure 6. Sorting *S. Typhimurium* cells from a mixture of *S. Typhimurium* and *E. coli*. (A) Epifluorescence image of a mixed culture of *S. Typhimurium* expressing GFP and *E. coli* expressing RFP. (B) Epifluorescence image of the sorted cells on silicon using a checkerboard microarray pattern of an antibody highly specific to the CFA/I fimbriae of *S. Typhimurium*.