Patterning of Solid Surfaces for Studies of Bacterial Adhesion and Communication

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INTRODUCTION

Bacteria are small, single-celled organisms which can be both helpful and harmful to humans. Many bacterial species are harmless to humans and can even be beneficial. However, multiple pathogenic species exist that are customarily killed with antibiotics. The recent emergence of antibiotic resistant bacteria have prompted the search for new methods of controlling bacterial growth and for better understanding their behavior.

A promising bacterial behavior is known as "quorum sensing." This is a complex bacterial behavior involving communication, and regulation[1]. Bacteria emit substances known as autoinducers, and a buildup in concentration of these autoinducers can lead to changes in genetic expression profiles. This can induce bacterial binding to surfaces, expression of toxins or virulence factors, and even increased pathogenicity. Typically quorum sensing has been studied in depth in situations where there are high concentrations of cells[1](typical cell culture conditions). What remains to be studied is if quorum sensing is observed at low cell concentrations and in different physical and chemical environments.

The goal of this effort was to create fixed micro-arrays of bacteria so quorum sensing can be explored at low cell concentrations at defined spatial distances. Previously there has been much experimental success with fixing proteins onto surfaces with molecular biological techniques. The challenge of this study is to develop micro-arrays of living cells so their life-cycles and behavior can be explored.

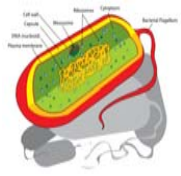


Figure 1. Schematic diagram of a typical bacterial cell

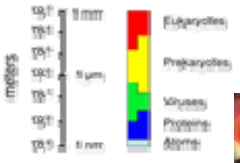


Figure 2. The relative size of prokaryotes

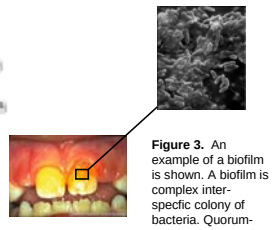


Figure 3. An example of a biofilm is shown. A biofilm is complex inter-specfic colony of bacteria. Quorum-sensing is key to the formations of these biofilms.

METHODS

Several methods were explored to attach bacteria to a surface in specific patterns. Central to achieving this goal was the use of the NanoEnabler™ from BioForce Instruments. This device works as a miniature "fountain pen" which allows to allocation of small amounts of fluids. Ultimately this provides selective positioning of proteins or antibodies that can bind to bacterial cells.

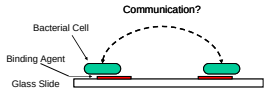
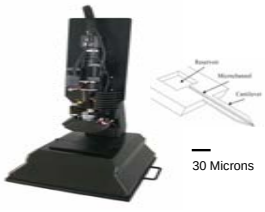


Figure 4. Small amounts of protein or antibodies deposited by the NanoEnabler will be used to attach bacterial cells to the surface.



The NanoEnabler™

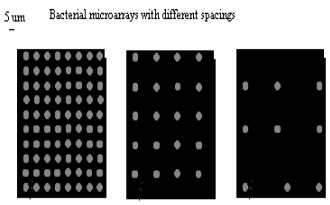
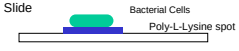


Figure 5. This an example of what a bacterial array may look like. Each Dot represents either a small group of bacteria or single cells. Differences in spacing could yield different responses to quorum sensing signals.

One of the major goals of this work was to develop an immobilization method for cells onto solid surfaces. Several theoretical methods were tested. Method I utilizes the printing of positively charged poly-L-lysine with the NanoEnabler onto a plain glass surface. Bacteria (*E. coli*) could then be exposed to the slide so that cells would bind to the poly-L-lysine spots. In Method II, plain glass slides were either coated in poly-L-lysine, or purchased with an aminosilane coating. These slides were then spotted with anti-*E.coli* antibody (using the NanoEnabler). The slides were then blocked with non-fat dry milk (NFDM) to prevent non-specific background binding. The slides were then exposed to bacterial cells. In a final method, Method III, the aminosilane and poly-L-lysine slides modified with glutaraldehyde (to promote a cross-linking between the slide surface and antibodies). Antibodies were printed with the NanoEnabler and by hand. The slide surface was blocked with NFDM and exposed to *E. coli* cells. The strain of *E. coli* used was modified to express the green fluorescent protein (GFP).

Two other procedures were used for quality control purposes. First to test if the antibodies were indeed bound to the surface, some slides spotted with antibody (Method III) were exposed to a secondary antibody (FITC anti-goat) instead of bacteria. This second antibody binds to the primary (anti-*E. coli*) antibody that is produced in goat. The FITC-tagged secondary antibody fluoresces bright green, allowing us to visualize spots were the primary antibody was successfully bound. In addition, we deposited anti-BSA antibodies onto glass surfaces and used FITC-labeled BSA to visualize antibody binding to its target.

Method I

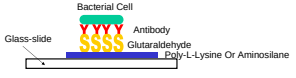


In this first method the bacterial cells were expected to bind to the areas where poly-L-lysine was printed by the NanoEnabler

Method II

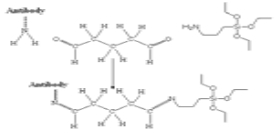
In the second method antibodies were printed with the NanoEnabler onto slides coated with poly-L-lysine or aminosilane. Specific binding of cells by these antibodies was expected.

Method III



In this third method, antibody-slide attachment is improved by soaking the poly-L-lysine or aminosilane slide in glutaraldehyde, a well-known crosslinker agent. This covalently binds amine groups on the antibodies and the aminosilane or poly-L-lysine slide surfaces.

Cross-Linking Chemistry of Method III



Above is a cartoon of the cross-linking effect between aminosilane and the antibody, mediated by the glutaraldehyde crosslinker.

RESULTS

Specific binding of antibodies was achieved with limited success. The first method (Method I) of trying to bind cells by spotting down poly-L-Lysine on a surface did not provide specific binding (Figure 6). The most likely reason for this is because the spotted Poly-L-Lysine relied on electro-static interactions and did not provide a fully adhesive covalent bond between the slide surface and bacterial cells. The second method (Method II) where anti-*E.coli* antibodies were printed onto aminosilane-terminated glass before a bacterial wash also did not provide specific binding. A possible reason for this was either that the antibodies were not covalently binding to the surface of the slide (amine does not bind to amine) or the cells were not binding to the antibodies (data not shown). Method III theoretically corrected one of these problems by cross-linking the anti-body covalently to the surface. After the bacterial wash, however, specific binding was still not achieved by NanoEnabler-printed antibody patterns . Limited bacteria binding specificity was achieved however when larger amounts of anti-body was deposited on the surface by hand (Figure 9).

The quality control steps provided helpful information about why the bacterial cells were not binding to the protein or antibody arrays. The printing of anti-BSA antibodies and cross-linking them to the surface of an aminosilane-terminated glass slide provided limited specific binding of FITC-tagged BSA (data not shown). In addition to these methods, plain glass slides coated with poly-L-Lysine produced significantly more background than the aminosilane-terminated slides (data not shown). However, neither of these methods provided specific cell binding when antibody was printed with the NanoEnabler.

The second quality control step provided additional information about our technique. Anti-*E.coli* antibodies were printed on the glutaraldehyde treated Aminosilane slides (poly-L-Lysine was abandoned because of intense background signal) and then blocked with nonfat dry milk (NFDM). Instead of being incubated with bacterial cells (as in Method III) the slides were instead incubated with FITC-labeled anti-goat antibodies (which could bind to the anti-*E. coli* antibodies). These secondary antibodies bound to the deposited anti-*E.coli* antibodies that had been cross-linked to the surface. This experiment showed that the anti-*E. coli* antibodies had been deposited and that the FITC anti-goat antibodies could bind to them, resulting in highly fluorescent spots (Figures 7 & 8). However, these *anti-E. coli* antibody arrays were not able to bind to *E. coli* cells. The reasons for this failure is unclear. One possible explanation is that that the antibodies are bound the surface in such a way that their antigen binding sites are not presentable to the cells.

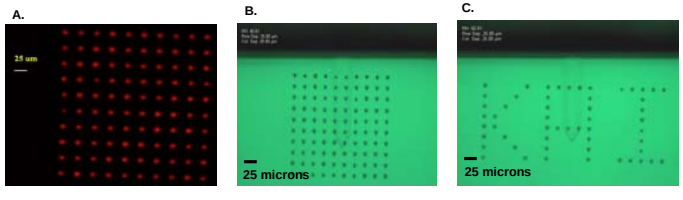


Figure 6. These images show successful printing of poly-L-lysine on a slide surface. Rhodamine red labeled poly-L-lysine was printed onto glass and imaged with an epifluorescence microscope. Panels B and C show unlabeled poly-L-lyisne as it is being printed onto glass surfaces (imaged with the NanoEnabler integrated camera system).

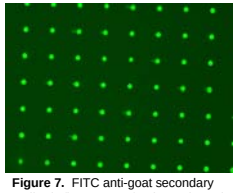


Figure 7. FITC anti-goat secondary antibody fluoresces after it was bound to the anti-*E.coli* antibody that had been printed with the Nano-Enabler.

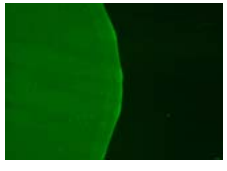


Figure 8. FITC anti-goat secondary antibody fluorescing after it has bound specifically to the anti-*E.coli* antibody that had been printed by hand (using a micropipettor).

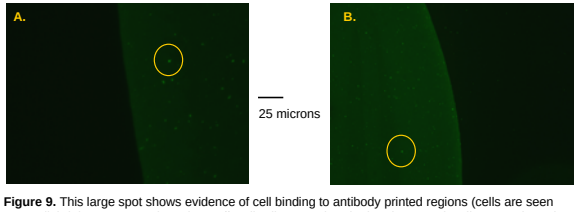
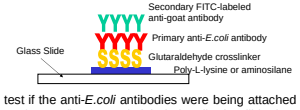


Figure 9. This large spot shows evidence of cell binding to antibody printed regions (cells are seen as small, bright green spots). Anti-*E. coli* antibodies were hand printed onto aminosilane terminated slides, modified with glutaraldehyde. The surface was exposed to *E. coli* cells and to FITC anti-goat secondary antibody to visualize both cells and the deposited antibody spots.

Quality Control Steps

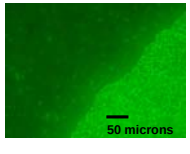
To provide further information about the crosslinking, a different type of antibody (anti-BSA) was printed by the same method as Method III. Instead of a bacterial antigen, the antigen is a florescently-labeled BSA protein.



To test if the anti-*E.coli* antibodies were being attached to the surface, a fluorescently-labeled antibody will be bound to the *anti-E. coli* antibodies. This anti-goat secondary antibody can bind to the anti-*E. coli* antibodies which were produced in goats.

FUTURE WORK

In the future, methods of specifically attaching cells to the printed antibodies must be optimized. This will be performed by optimizing array deposition conditions, as well as through the use of alternative antibodies and target cells. We will also use alternative solid supports such as nylon and PVDF. Once these procedures have been optimized, we will see to explore quorum sensing behavior at low cell densities in controlled intercellular distances. With successful cell patterning methods, the study could also be extended to mammalian cells for studies of cell signaling in other species. The ability to spatially pattern cells opens up great potential for many future studies of cellular behavior.



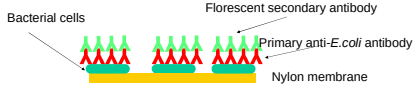


Figure 10. A test to assess the quality of antigen-antibody binding. Preliminary work has been successful in attaching antibodies to immobilized cells (left).

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