Polymer Microstructures for Multianalyte Protein Assay

by

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Abstract

This paper describes the use of a laser-based method to create microstructures within a bilayer system of gold and a non UV-sensitive polymer, poly(methyl methacrylate). Patterned surfaces were achieved through the use of a computer-controlled laser exposure system, comprising a research-grade inverted optical microscope, a pulsed nitrogen laser emitting at 337 nm and a programmable X-Y-Z stage. Those microstructures have proven to be useful for the adsorption of a variety of proteins. The technology has also been applied to an antigen-antibody specific recognition experiment, which proved the feasibility of this system for multianalyte protein assay. In the future, this system will be used as a platform for the optimisation of cell-cell communication within functionalised microstructures. In particular, the functionalisation of these micro-channels with neuropeptides is foreseen, with the aim of investigating effects that influence nerve regeneration.

1. Introduction

Antibody-based microarrays are thought to be only the first-generation of protein-based micro-devices, which are to become versatile and powerful tools for various molecular and cellular analyses including clinical diagnostics and complete protein complement of the genome.[1-3] Protein arrays are currently under intensive exploration, but many critical aspects of their fabrication (e.g., protein-surface interaction) and functions (protein-protein interaction) are grossly underestimated when an analogy with DNA arrays is done. [4] In respect of rapidity and moderate cost biosensing systems offer an attractive alternative to the existing methods allowing rapid, efficient, and quantitative protein detection.[5-8] Most technologies for the fabrication of protein chips have to ensure the confinement of different protein molecules in localised areas, flat, 2D, or profiled, '2D+' micro-areas. Among the technologies available for the fabrication of protein chips one can mention (i) spotted-array-based methods; [9-10] (ii) soft lithography; [11-12] (iii) photolithography; [13-16] (iv) scanning probe lithography; [17] (v) laser or ion-beam ablation; [18-19] and (vi) microfabrication of profiled features, for example, microfluidic devices. While some types of biodevices dictate a particular design of the biodevices (eg. microfluidics devices will ask for profiled channels) others do not (e.g., microarrays would have normally a flat surface). The profiled features have the advantage of minimisation of inter-spot contamination and the drawback of more difficult access of the recognition biomolecule (e.g., antigen for antibody microarray) in a micro-confined area, but an optimal shallow profile feature would take advantage of the benefit of the former and mitigate the latter.

Among the enabling technologies for the above biomolecule micropatterning methods, laser beams are capable, according to the exposure energy and the sensitivity or adsorbance of the exposed material, to enable both photolithography and photo-assisted etching. Also, focused laser beams can solve, in principle, a critical fabrication and operating problem of the protein chips better than most other alternative methods, ie. the controlled and confined variation of the surface properties of the micro-areas where different proteins are deposited on. In contrast to the simpler DNA molecules, proteins present extremely various molecular surfaces (e.g., hydrophilic or hydrophobic; acidic or basic; neutral or charged) that interact with the surface via electrostatic forces, hydrogen-bonding, Van der Waals or hydrophobic interactions. This variety of molecular surface – biochip surface interactions can lead to large variations in protein surface concentrations as well as possibly important changing of the protein bioactivity and its denaturation. The present work reports on the fabrication via laser ablation of shallow-profiled microstructures with surface properties of the microlines tailored to accommodate different types of proteins through physical adsorption. Moreover, the microstructures were used for specific antibody-antigen recognition.

2. Microstructures for Multianalyte Protein Assay

2.1 Film Preparation and Microstructure Fabrication Through Laser Ablation

Linear microstructures were fabricated through a localised laser ablation of a protein-blocked thin gold layer (50 nm) deposited on a Poly(methyl methacrylate) film. The laser-based microfabrication of gold-coated polymeric films was accomplished using a fabrication platform that consists of a computer-controlled laser ablation system, comprising a research-grade inverted optical microscope, a pulsed nitrogen laser emitting at 337 nm and a programmable XYZ stage (Figure1). The micro-lines were created using the 40x objective, which lead to the fabrication of 5 μm width features. The deposition of the proteins was done using a pico-liter pipette mounted on the same fabrication platform, which permitted the deposition of approximately 100 pL. In order to recognise different type of proteins, when ablating the gold layer, a combination of vertical lines were used, giving a 'bar code', 'informationally-addressable' mode and not a 2D, spatially-addressable mode like in the classical microarrays.

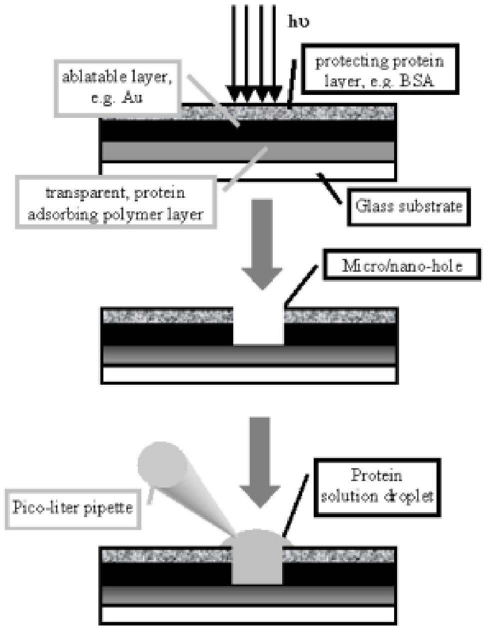

Figure 1 - Procedure for the fabrication of micro-wells for protein solution droplets.

AFM studies revealed that the micro-ablation of gold induces local chemical and physical changes in the top surface of the polymer as well as a higher specific surface, which cooperate to achieve a higher and a reproducible surface concentration of proteins in micro-lines.

2.2 Protein Adsorption

The adsorption of different proteins was studied. For istance, the adsorption of an antigen (human IgG) and a globular protein (hen egg-white lysozyme) was studied. Both proteins were labelled with AlexaFluor 456 before deposition and had the same concentration in solution (0.14 μg/ml). Samples were analysed using a fluorescent microscope. The difference in adsorption was inferred from the difference in fluorescence of the two proteins.

2. 3 Antigen-Antibody Recognition

One of the most attractive potential of this technique is to specifically recognise a protein within a mixture of it. This protein detection system was demonstrated by a microarray having a 'bar code' microstructure that was functionalised with two proteins (an antigen and streptavidin, a globular protein) both not fluorescent. The chicken IgG antigen was deposited on a fragment of the 'bar code' microstructure (three lines on the right, Figure 2, (a)) whereas streptavidin was deposited on the rest of the microstructure (two lines on the left, Figure 2, (a)) using the picoliter pipette. The area was than flushed with a fluorescent solution of chicken-antibody IgG (labelled with Alexa Fluor 456). The fluorescence of the three lines on the right of the bar code in Figure 2 (b) demonstrates that the chicken-antibody IgG specifically recognised the corresponding antigen.

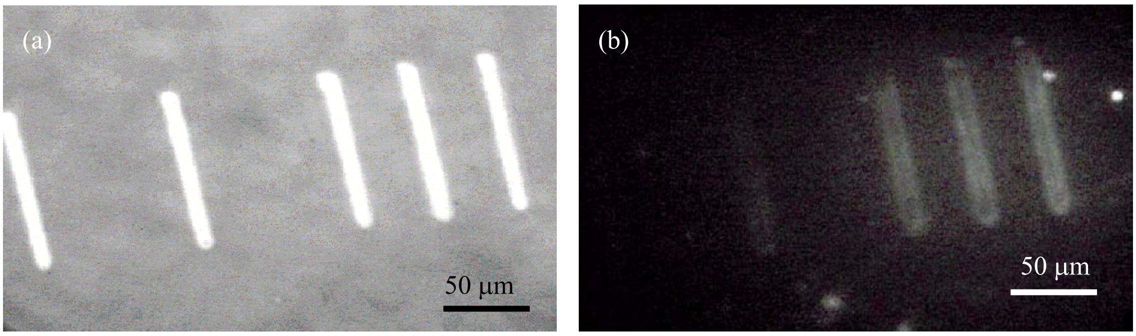

Figure 2 - (a) IgG-streptavidin bar code visualised through a bright field (no fluorescence); (b) fluorescent image of the same bar code: the fluorescent lines correspond to the antigen-antibody recognition.

3. Functionalised microstructures for cell-cell communication

The system so far described can be used as a platform for cell-cell communication due to the suitable dimension of the features presented. Also, these microstructures can be functionalised to specifically address cell-cell communication. One of the future application foreseen, for example, is the functionalisation of such microstructures with neuropeptides and other specific biomolecoles.

4. Conclusions

In this work a novel method for the fabrication of microstructures via the selective ablation of an ablatable thin layer (such as 50 nm gold) deposited on top of a polymeric surface that promotes protein adsorption (such as Poly(methyl methacrylate). Linear microstructures both decrease the actual amount of protein used for deposition and increase the capacity for miniaturisation in a lateral non-2D manner. Also this approach opens the possibility to encode the information (e.g., type of antibody, concentration) through a combination of vertical lines in a ‘bar code’, and to use the microstructures for multianalyte protein assay, such as antigen-antibody recognition. The system described can be used as a platform for cell-cell communication and the use of it in the study of nerve regeneration through the use of functionalised microstructures is foreseen.

5. References

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