Amplification of protein adsorption on micro/nanostructures for microarray applications

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ABSTRACT

The fabrication and operation of biodevices require the accurate control of the concentration of the bioactive molecules on the surface, as well as the preservation of their bioactivity, in particular proteins that have a significant propensity for surface-induced denaturation. A method that allows the adsorption of proteins on 'combinatorial' micro/nano-surfaces fabricated via laser ablation of a thin metal layer deposited on a polymer that has been proposed allows for the up to 10-fold amplification of the protein adsorption on micro/nano-structures. This contribution studies the relationship between amplification of adsorption and protein molecular characteristics (total molecular surface; and charge- and hydrophobicity-specific surface).

Keywords: microarray, protein array, protein adsorption, microstructures, nanostructures

1 INTRODUCTION

Protein based microdevices have a long tradition, e.g. immunoassays, but the emergence of proteomics brought them in the center of attention of the scientific community. Compared with the classical protein-based devices, the new protein micro- and nano-arrays pose important technological challenges arising from (i) the large variety of proteins needed to be immobilized on the surface, in contrast with the rather similar antibodies; (ii) increased density of laterally defined areas with specific immobilization for specific proteins, required by high-throughput analysis; and (iii) the ever present complexities of protein-surface and protein-protein interactions, reflecting in complex fabrication and function, respectively. For all these reasons, the simple extrapolation of the technology responsible for the precursors of protein micro/nano-arrays, although attractive, is unwarranted or at least questionable.

Most technologies for the fabrication of protein chips have to ensure the confinement of molecularly different proteins in laterally-defined, either flat 2D; or profiled '2D+' micro-areas. The profiled features have the advantage of minimization of inter-spot contamination and the drawback of more difficult access of the recognition biomolecule (e.g. antigen for antibody microarrays) in a micro-confined area. Recently we have proposed a method for protein immobilization in micro/nano-channels fabricated via laser ablation of a thin metal layer deposited on a polymer immobilization substrate. The shallow profiles of the microfabricated features are expected to take advantage of the benefit of the flat areas and mitigate the drawback of the deep structures.

The present study investigates the adsorption of five, molecularly different proteins on micro/nano-structures fabricated via laser ablation, to probe the relationship between the amplification of the protein adsorption and their molecular characteristics (total molecular surface; and charge- and hydrophobicity-specific surface).

2 METHODS

The adsorption of five proteins with very different molecular characteristics, i.e. α-chymotrypsin, human serum albumin, human immunoglobulin, lysozyme, and myoglobin, has been characterized using quantitative fluorescence measurements and atomic force microscopy. The procedure for the fabrication of micro/nano-structures, which has been used for this study, has been described elsewhere [1]. Briefly, the process consists of (Figure 1) (i) the glass slides covered a thin polymer layer of Poly(methyl methacrylate) –PMMA- were covered with a thin (~50nm) layer of gold (sputtered); (ii) the gold-layered substrata were then incubated with bovine serum albumin (BSA); (iii) the gold and blocking protein layers were ablated (due to the opacity of the gold) with a laser (Cell Robotics workstation, 337nm, 20pulses/s, 10ns/pulse); and (iv) the proteins were deposited either by flooding the whole surface with a protein solution (protein attaches on PMMA-surfaces opened by ablation); or by spatially addressable deposition with a picoliter pipette mounted on a xy motorized table.

The molecular descriptors of the selected proteins have been calculated using an in-house developed program (freely downloadable [2]), that uses the Connolly algorithm beyond its original purpose (i.e. the calculation of molecular surface) for the calculation of the surface-related molecular properties (i.e. surface positive and negative charges; and surface hydrophobicity and hydrophilicity) as well as the molecular surfaces related to these properties.

Atomic Force Microscope (AFM) was used to characterize the topography and the relative hydrophobicity of the micro/nanostructures. The relative level of adsorption was quantified using a fluorescence reader.

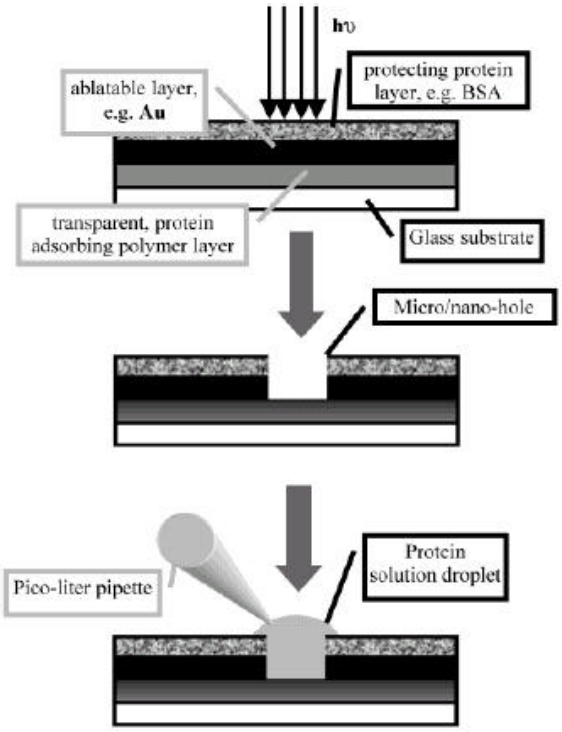

Fig 1: Fabrication of micro/nano-structures for protein arrays using microablation and directed deposition.

3 RESULTS AND DISCUSSION

The proposed technology produces surfaces that present to the proteins a large variation of the properties (in particular hydrophobicity and rugosity) of the surface concentrated in a small, micron-sized region. The ablation of the thin metallic layer induces the pyrolysis and partial 'sculpturing' of the polymer, with more hydrophilic surfaces towards the edges of the channel and a hydrophobic hump in the middle. The higher rugosity of the microstructures (Figure 2) translates in a 3 times more specific surface in than outside the channels.

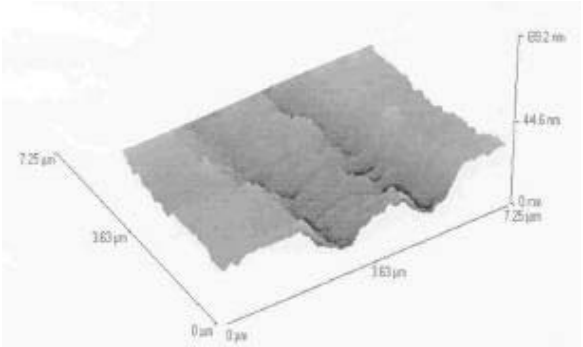

Fig 2: AFM mapping of the ablated micro-channels.

A more important feature than the increased rugosity in the microablated areas is the large variations in the relative hydrophobicity of different regions, as measured by AFM in lateral force mode. Fig 3 presents both the topography and the relative hydrophobicity of the surface of microablated lines.

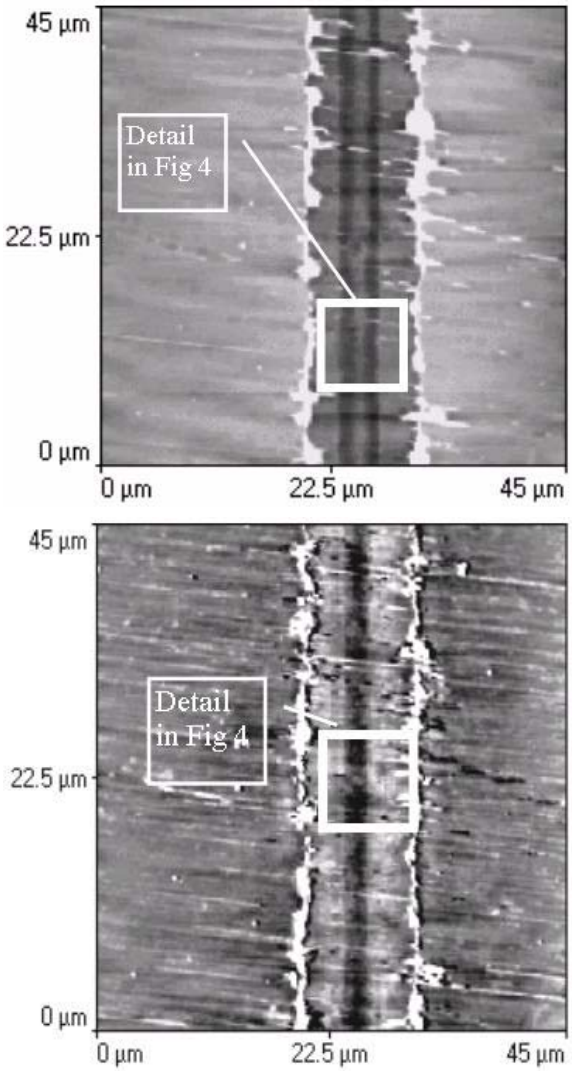

Fig 3: Topography (top) and relative hydrophobicity (bottom) of the surface of the microablated lines. Darker regions represent deeper levels and hydrophobic surfaces, on the topography and lateral force images, respectively.

These structures have micron-size dimensions laterally but tens of nanometers in depth. The latter dimensions make the structures comparable with medium to large proteins (Fig. 4).

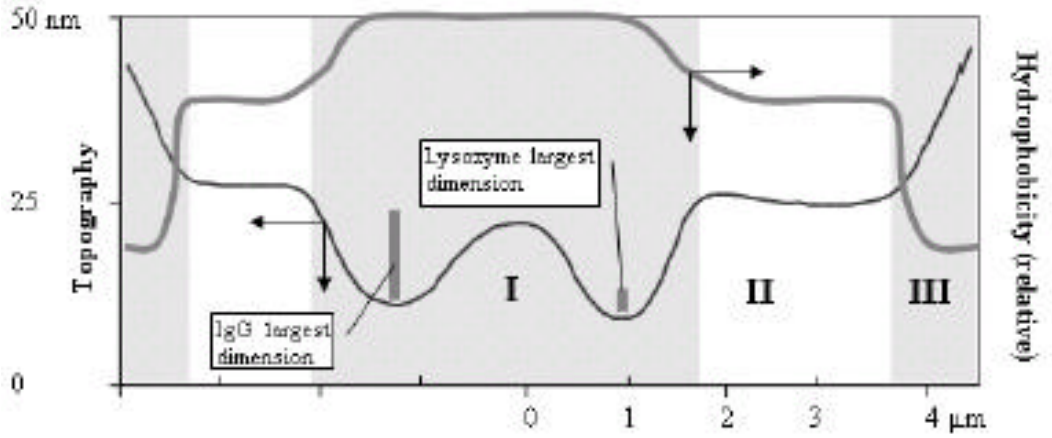

Fig. 4: Typical lateral distribution of topography and hydrophobicity in microablated channels. The largest dimensions of lysozyme and IgG are depicted for comparison.

Although there are variations of the dimensions and distribution of hydrophobicity vs. laser power, the general structure of the microablated channels remains the same. Although the process can use different polymers and metals, we found that PMMA (and gold) are so far optimum choices. In particular PMMA can offer large possibilities to 'combinatorialise' the chemistry of the surface upon thermolysis. Figure 5 depicts possible chemical pathways that would explain the AFM-measured variation in hydrophobicity.

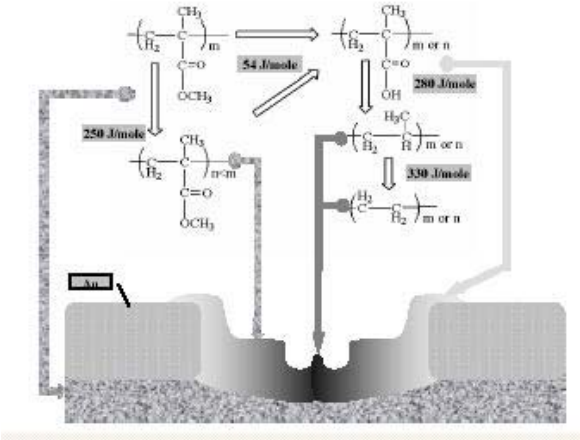

Figure 5: Possible pyrolysis pathways of PMMA localized in micro-regions leading to the observed lateral distribution of hydrophobicities.

The strategy behind this method was to allow different proteins, or different parts of the same large protein, to find the most appropriate –in terms of adsorption and preservation of bioactivity- surface. Because the different micro/nano-surfaces are co-located in a small area (channel width around 10 µm or less) the florescence signal from the proteins would be perceived at the mm-range as being located in the same areas. Also it has been observed that the concentration of the proteins was apparently higher in the microstructures than on flat areas with similar material due to a higher specific surface and larger opportunities for attachment.

The combinatorial character of the surface would in principle also allow the probing of several patches on the molecular surface of the proteins, or modulate their bioactivity. Fig. 6 presents possible arrangements of IgG-like biomolecules on the combinatorial surfaces.

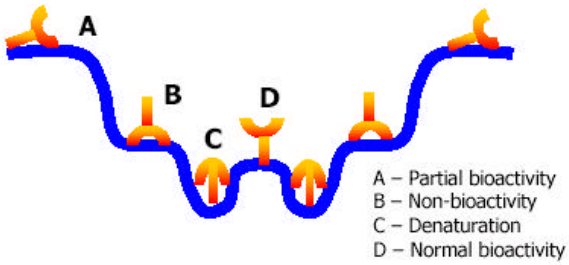

Fig. 6: General concept of the probing of molecular surface of proteins.

The most important observation relates to the correlation between molecular properties of the adsorbed proteins and the amplification of their adsorption. For instance, it was also observed that the amplification of the fluorescence signal (due to increase of adsorption) is larger for smaller proteins (e.g. lysozyme) than for larger proteins (e.g. IgG). The proteins with a molecular surface in the order of tens on nm^2 adsorb up to 10-12 times more on microstructures than on flat surfaces, while large proteins with a molecular surface of few hundreds of nm^2 adsorb only 3 times more than on bare PMMA flat surface. Figure 7 presents synthetically this relationship.

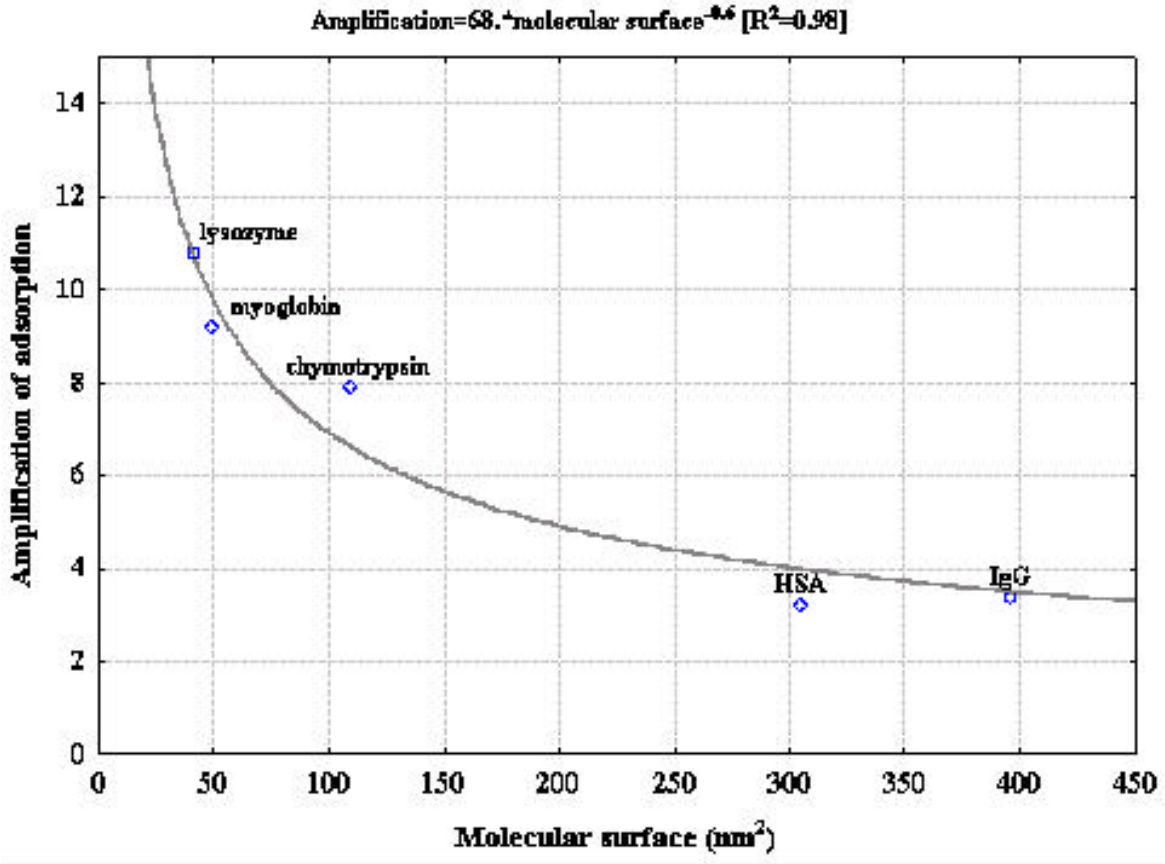

Fig. 7: Amplification of adsorption vs. molecular structure of the tested proteins, i.e. lysozyme, myoglobin, α-chymotrypsin, HAS and IgG.

The proposed technology for the fabrication of microarrays has the following potential advantages: (i) the combinatorial surfaces would improve the uniformity of biomolecule surface concentration, in particular for protein microarrays; (ii) the surface concentration of biomolecules (several very different proteins being tested) increases, and therefore the sensitivity increases accordingly, by 3-12 times, depending on the molecular properties of the biomolecules; (iii) it is possible -in principle- to probe different sides of the biomolecular surface and therefore the bioactivity;m and (iv) because the proposed method writes protein lines (that can encode information in a bar code manner) instead of dots, it can be used for the fabrication of informationally-addressable, as opposed to spatially-addressable microarrays.

4 CONCLUSION

We propose a method for the fabrication of random 'combinatorial' micro/nano-sized surfaces that allows a higher adsorption of proteins and possibly the immobilization of biomolecules on different sides of the molecular structure. The method, which is based on laser microablation of thin metal/blocking protein layers deposited on a polymer substrate, has proven to amplify the protein adsorption between 3 to 10 times.

ACKNOWLEDGEMENTS

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