PROTEIN MICROARRAY ON CYCLIC OLEFIN COPOLYMER (COC) FOR DISPOSABLE PROTEIN LAB-ON-A-CHIP

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ABSTRACT

This paper presents a novel method of high density protein microarray patterning on Cyclic Olefin Copolymer (COC) surfaces for the development of disposable protein lab-on-a-chip for proteomics applications. Protein microarrays have been generated via photolithography and plasma treatment and then fully characterized for Human-IgG. In addition, the protein adsorption of COC was compared with that of other polymer materials such as Polycarbonate (PC) and Polymethyl-Methacrylate (PMMA), which are known as good materials for biochips.

KEYWORDS: Lab-on-a-chip, protein microarray, surface modification, immunoassay

INTRODUCTION

Protein chip or protein microarray is a versatile product that will be able to analyze thousands of samples simultaneously to diagnose diseases at the molecular level at low cost, in a short time and with high performance. Protein patterning is a physically defined form of protein immobilization. So the choice of substrate material is important for making protein chip. Recent research for polymer substrates for biotechnology applications will offer easy fabrication of 3-dimensional structure, ease of surface modification, low cost and disposable protein lab-on-a-chips. At µTAS 2002 [1] we had reported the original concept of protein adsorption on treated COC surface. In this paper, we have compared the protein immobilization on other polymers and expanded the basic concepts of protein adsorption on COC to the patterning of high density protein microarray by using lithography techniques.

MECHANISMS OF PROTEIN ADSORPTION FOR PATTERNING

Since IgG molecules are the most abundant of circulating antibodies [2], Human-IgG and its anti-IgG conjugated with FITC (excitation peak at 495 nm and emission peak at 535 nm) have been chosen to study protein adsorption and patterning. The protein (Human-IgG) adsorption was evaluated by intensities of anti-Human-IgG conjugated to FITC. Stock concentration of Human-IgG was diluted with PBS (phosphate buffer solution), and at low concentrations, the intensity was used for measuring Human-IgG...
adsorption. As Figure 1 shows that the adsorption of Human-IgG on COC surface increases linearly with protein concentration. We used a 0.02 mg/mL of IgG solution to investigate adsorption properties. As reported in our previous work [1], the COC surface can be modified by a combination of O₂, N₂, and CF₄ gases using RIE and the resultant change in surface properties can be characterized by contact angle. Figure 2 shows that protein, Human-IgG, is likely to adsorb well on hydrophobic surfaces compared with hydrophilic surfaces. That is because most of bonding strength between the protein and polymer surface is determined by the hydrophobic force. This property can be used to make high density protein arrays by making hydrophobic spots in micrometer range on a hydrophilic background using conventional photolithography and surface modification by RIE.

PROTEIN ADSORPTION ON VARIOUS POLYMER SURFACES

Injection molded polymers [3], COC (Cyclic Olefin Copolymer), PC (Polycarbonate), and PMMA (Polymethyl methacrylate), which all are biocompatible polymers used widely in biotechnology fields, have been studied for optical transparency as shown in Figure 3 [4]. Figure 3 shows that COC has better optical transparency in UV range which is important for optical detection of biomolecules.
Figure 4 shows fluorescence intensities of adsorbed Human-IgG on various surfaces. COC surface display a high protein binding capacity. Considering high glass-transition temperature, high decomposition temperature and low moisture adsorption, COC has been chosen as a substrate for protein microarray patterning and protein biochip.

PROTEIN MICROARRAY PATTERNING

Patterning of protein microarray is illustrated in Figure 5. Photoresist (S1818) was used to make an array of spots (200 μm in diameter and spacing) using conventional photolithography, followed by O₂ plasma. After the photoresist is removed, a hydrophilic plastic slide with hydrophobic spots was formed. The treated COC slide was immersed in 0.02 mg/mL Human-IgG solution, incubated for 45 minutes, rinsed in DI water, and dried with nitrogen. Then the chip was immersed in 0.02 mg/mL FITC labeled anti-Human-IgG for 30 minutes, rinsed in DI water, and dried with nitrogen. Finally the slide was scanned by Typhoon® 8600 fluorescence scanner.

Figure 5. Patterning of COC surface using oxygen based RIE treatment and the resultant localized binding of IgG.

Figure 6. Scanned image of protein microarray with spot size of 200 μm in diameter and spacing, respectively.

Figure 6 shows a microphotograph of the high density protein microarray which has been obtained by the method above. This approach allows considerable flexibility in protein chip design. The minimum size is limited by the resolution of the scanner. For the fabrication aspect, it is easy to scale the spot size down to 10 μm in diameter and spacing by using conventional photolithography tools. This is a significantly higher spot density than achievable by commercially available precision robotic spotters. Furthermore, the low cost plastic substrate can potentially be integrated with a microfluidic system for developing an integrated disposable protein chip.
CONCLUSION

In this work, a novel technique for generating high-density protein arrays has been developed and characterized. The new technology uses established microlithography tools and has the potential to generate low cost, high density protein array chips. We have also characterized three polymer substrates: PC, PMMA and COC for the protein chip application. These results will be of great relevance for the realization of disposable protein lab-on-a-chips in low-cost for genetics and proteomics.

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REFERENCE


