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Materials Science and Engineering C 24 (2004) 151–155
MATERIALS SCIENCE & ENGINEERING C
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Pattern formation of cytochrome *c* by microcontact printing and dip-pen nanolithography

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Abstract

We report the results of fabricating micrometer and submicrometer-scale patterns of cytochrome *c* on gold surfaces. We used direct microcontact printing (μCP) and indirect dip-pen nanolithography (DPN) for fabricating cytochrome *c* arrays. The protein dots were formed in diameters of 2 μm by μCP and of ~ 200 nm by DPN, respectively. We analyzed the pattern size and height of protein arrays with atomic force microscopy (AFM). We expect that these methods will be potentially useful for developing small-scale biosensors and protein chip microarrays.

Keywords: Protein array; Cytochrome *c*; μCP; DPN; AFM

1. Introduction

Arrays of biomolecules immobilized on solid surfaces are relevant to many areas of science and technology [1]. Surface-bound molecules find applications in biosensors, chromatography, cell culturing, DNA microarrays, and other analytical procedures. Many conventional patterning techniques, including photolithography and spot arraying, have been used for fabricating such arrays of biomolecules [2,3]. Recently, micro-contact printing (μCP) and dip-pen nanolithography (DPN) have been developed as patterning techniques.

μCP is a flexible method that routinely forms patterned self-assembled monolayers (SAMs) containing regions terminated by different chemical functionalities with micrometer-scale lateral dimensions [4,5]. An elastomeric polydimethylsiloxane (PDMS) stamp is used to transfer molecules of the "ink" to the surface of the substrate by contact. This technique is advantageous because it is simple and easy to use even without complicated tools and facilities.

DPN is to deposit molecules having chemical affinity with the substrate by using dip-coated AFM tips [6]. Directly written patterns are formed by capillary transport of molecules from the AFM tip operated in air to the substrate. This technique is a useful tool for fabricating submicrometer or nanometer-scale patterns on the surfaces of various substrates [7–9].

In this study, we demonstrate fabrication of protein patterns on gold by choosing cytochrome *c* as a protein since it has been widely studied in many research fields owing to its unique physiological and physicochemical properties. For instance, the cytochrome *c* has a capability of electron transport that is of much interest in the application to biomolecular photodiode systems [10,11].

We fabricated protein dot arrays of the cytochrome *c* in two ways: First, the protein arrays in micrometer-scale were directly transferred from a PDMS stamp by μCP over the self-assembled monolayer of alkanethiol molecules on gold. Second, the submicrometer-scale arrays were formed by protein adsorption from solution to the patterned self-assembled monolayer produced by DPN on gold. Both results will be helpful for developing bioelectronic devices in the future.

2. Experimental

2.1. Materials

16-Mercaptohexadecanoic acid (MHA, $HS(CH_2)_{15}COOH$; 95+%) and 1-octadecanethiol (ODT, $HS(CH_2)_{17}$

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doi:10.1016/j.msec.2003.09.015