Nonbiofouling Patterned Surface for Obtaining High Signal/Noise Ratio in Microarray Biosensors

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Statement of Purpose: To create useful materials for many biotechnology applications, interfaces are required that have both enhanced specific binding and reduced non specific binding. Thus, in application such as biosensors, the tailoring of surface chemistry and the use of micro or nanofabrication technique becomes an important avenue for the production of surface with specific binding properties and minimal background interference[1] especially in microfluidic devices handling very low concentration and small amount of volume of analyte. The aims of this study are to prepare nonbiofouling surface bearing highly biocompatible poly(2-methacryloyloxy-ethyl phosphorylcholine (MPC)) brushes[2] and to construct micropatterned recognition layer on microfluidic system for enhancing high signal/noise (S/N ratio) in biosensors (Figure 1) using living radical polymerization based on diethyldithiocarbamate as photoiniferter (initiator, transfer, and terminator)[3].

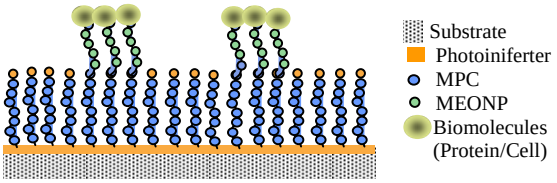

Figure 1. Illustration of micropatterned recognized layer construction over nonbiofouling poly(MPC) brush surface

Methods: Macrophotoiniferter comprised of 2-ethylhexyl methacrylate(EHMA) and 4-vinylbenzyl N,N-diethyl-dithiocarbamate(VBDC) (PEV) were synthesized with variation of VBDC content of 10% - 40% (abbreviated as PEV10, 20, 30, 40). Ultrasonically cleaned polymer substrates {poly(ethylene terephthalate)(PET), polycarbo-nate(PC) and poly(methyl methacrylate)(PMMA) were coated by dipping in 0.25% (by weight) of the macrophotoinifeter solution in toluene or 1,4 Dioxane. The photoinifeter-coated plates with degassed aqueous solution of 0.3 M MPC were irradiated with UV lamp (UVL-400HA, RICO, Chiba, Japan; 365 nm) at room temperature with variation of time. The surface characterizations of the modified surfaces (represented as PEV-g-MPC) were conducted by water contact angle (CA-W, Kyowa Interface Science Co, Saitama, Japan), X-ray photoelectron spectroscopy (Kratos Analytical, AXIS His 165 and ULTRA, Manchester, England), ellipsometry, and atomic force microscopy (Nanoscope IIIa, Veeco Instrument Inc., CA, USA). Amount of total non specific protein adsorption was determined by contacting the modified surfaces to a mixture of protein solution (Fibrinogen 0.03 g/100mL + Albumin 0.045 g/100mL in PBS, pH 7.1) for 1 hour at 37°C and the amount of total adsorbed protein was determined by Micro BCA method.

Results/Discussion: The density and length of chain of the poly(MPC) brushes were controlled by the compositi-on of VBDC in the macrophotoiniferter and irradiation time, respectively. As surface-confined photoiniferter are exposed to ultraviolet light in the presence of MPC, poly(MPC) chains polymerize from the surface by so called `grafting from` approach. The thickness and molecular weight of the poly(MPC)-grafted layer increased by irradiation time which are 95±14 nm and 320 kDa, respectively, after 3 hour irradiation. The hydrophilicity of the modified surfaces is greatly improved as can be seen from the decreasing of water contact angle from more than 88° to less than 30° after one hour irradiation indicating complete covering of the surface. The value of P/C (from XPS) increases by irradiation time and density of photoiniferter reaching at 0.07 which is close to theoretical value of 0.09. These indicate that the poly(MPC) was highly oriented on the surface. The topography and roughness of poly(MPC)-modified surface vary depends on the density of photoiniferter and irradiation time (Figure 2). Beside the hydrophylicity, these surface morphologies seem very dominant in determining the protein adsorption resistance as can be evaluated from the total non specific protein adsorption (Figure 3).

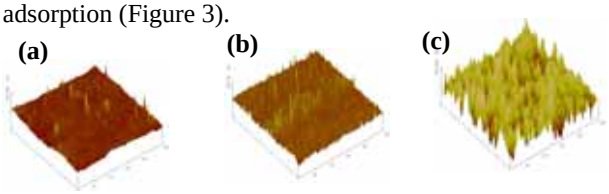

Figure 2. 3D topography of PEV30-g-MPC_{1h} (a), PEV20-g-MPC_{3h} (b), and PEV40-g-MPC_{3h} (c) with rms roughness of 6.40 nm, 9.86 nm, and 35.79 nm for a, b, and c respectively (images were taken of 50 μm x 50 μm in wet condition)

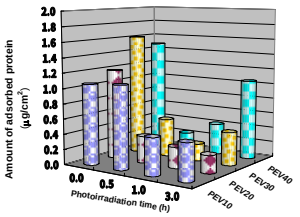

Figure 3. Total amount of non specific adsorbed protein

Conclusions: Preparation of nonbiofouling surface for microfluidic devices has been successfully conducted. One hour irradiation time and moderate chain density are effective in repelling non specific protein adsorption. Elimination of the non specific adsorption will lead to the high signal/noise in microarray biosensors.

References:

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3. Otsu T., Makromol. Chem. Rapid Commun., 1982;3:127-132