Extended Abstracts of the 2001 International Conference on Solid State Devices and Materials, Tokyo, 2001, pp. 448–449

A-7-1

Creation of High Performance Biocompatible Surface

Yukio Nagasaki[1], Chie Kuwahara[1], Mitsuhiro Kaneko[1], Kazunori Emoto[1], Yoshitsusgu Akiyama[2], Hidenori Otsuka[2], Kazunori Kataoka[2]
1. Department of Materials Science, Science University of Tokyo, Noda 278-8510, Japan
Tel: +81-471-24-1501; Fax: +81-471-23-8878; e-mail: Nagasaki@rs.noda.sut.ac.jp
2. Department of Materials Science, Graduate School of Engineering, The University of Tokyo, Bunkyo-ku Tokyo 113-8656, Japan

1. Introduction

Recently, an integration of mechanical, electrical and optical functions in a small domain such as silicon chip has been attracting in the field of micromachine technology. Such a fabrication is promising not only for Micro-Electro-Mechanical Systems (MEMS)[1] but also biological applications such as DNA and protein chips and micro Total-Analytical-System (μTAS)[2]. When the microfabricated structure is constructed for the biological applications, however, an effects of interface between device and biomolecules such as protein and cells increases significantly.

Surface modification with polymers is the most common way to control the surface properties for biological and biomedical applications[3]. Poly(ethylene glycol) (PEG) coating has been used to minimize non-specific fouling of materials surface with biocomponents, particularly plasma proteins. For example, a PEGylated surface which means the surface attached with the tethered chains of PEG using the functionality of PEG end groups extremely reduce protein adsorption [4] resulting in the high blood compatibility. PEG-coating can be performed in a variety of methods such as covalent grafting of PEG having reactive chain-end to the surface, graft copolymerization of PEG macromonomer with the surface and direct adsorption of PEG onto surfaces in the form of a surfactant or block copolymer in which one of the blocks is a PEG. Most of the PEG-coated surfaces, however, possess no reactive group on the PEG chain end. In order to provide the further functionality on the PEG-coated surface, several types of PEG derivatives were designed. This paper communicates synthesis and surface modification using several types of PEGs as surface modification agents.

2. Experiments

Synthesis of PEG derivatives for surface modifications.

CHO-PEG-SH: To dry THF (30 ml) in a 100 ml flask with a three-way stop cock under argon atmosphere, 3,3-diethoxypropanol (2 mmol) and potassium naphthalene (2 mmol) were added to form potassium 3,3-diethoxypropanolate (PDP). After stirring for 10 min, liquid EO (140 mmol; cooled below 0 ℃) was added via a cooled syringe to the mixture. The mixture was allowed to react for 2 days at room temperature. Then, alkolate group at the α-chain end was converted to methanesulfonyl group by the addition of excess amount of methanesulfonyl chloride as follows: the reaction mixture was added to dry THF (5 ml) with 40 mmol of methanesulfonyl chloride at room temperature and stirring for 6 hours, followed by the pouring into 1,000 ml of diethyl ether to obtain the polymer as white precipitate. Obtained polymer was dried *in vacuo*. The obtained acetal-PEG-SO_2CH_3 was reacted with potassium-*O*-ethyldithiocarbonate in a dry THF/DMF (24:1) co-solvent (25ml) as follows : To the obtained dry acetal-PEG-SO_2CH_3 (0.10 g), the 20-fold amount of potassium *o*-ethyldithiocarbonate in 25 ml of THF was added and stirred for 3 hours at room temperature. The reaction mixture was then mixed with chloroform and washed with a saturated NaCl aqueous solution several times to eliminate impurities from the polymer sample. Then, the organic phase was concentrated by evapolation after dried with sodium sulfate, followed by the pouring into diethyl ether. The resultant white precipitate was collected and was dried *in vacuo*. Finally the obtained acetal-PEG-S-C(=S)OCH_2CH_3 was converted to mercapto-end group by and amine treatment.

CHO-PEG/poly(lactide)(PEG/PLA): Acetal-PEG/PLA block copolymers have been synthesized by a one-pot anionic ring opening polymerization of EO followed by LA initiated with potassium 3,3-diethoxypropanolate (PDP) as an initiator at room temperature under argon. After the preparation of acetal-PEG-OK in THF as described above, 20 mmol (10.1 mL) of an LA solution in THF was introduced, and the polymerization proceeded for 90 min. The polymer was recovered by precipitation into a 20-fold excess of cold isopropyl alcohol (-15 °C), stored for 2 hours in the freezer and centrifuged for 30 min at 6,000 rpm. The polymer was then freeze-dried with benzene and the yield of the obtained polymer was ca. 90%.

Core polymerized CHO-PEG/PLA micelle: The core polymerized CHO-PEG/PLA micelle was prepared using CHO-PEG/PLA-methacryloyl block copolymer in aqueous media. The block copolymer was prepared in the way similar to CHO-PEG/PLA block as described

above. After the preparation of the acetal-PEG/PLA in THF, 20-fold of methacrylic anhydride was added to the reaction mixture. The polymer micelle was prepared by the dialysis method.

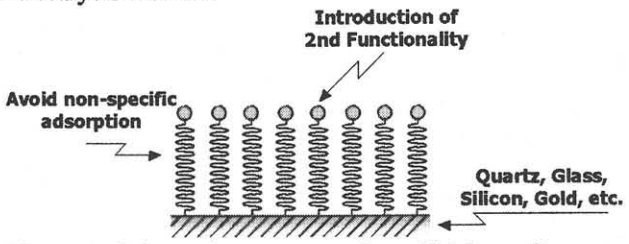

Figure 1. Schematic representation of high-performance PEG tethered chain interface

3.Results and discussion

The objective of our study was to construct end-functionalized PEG tethered chain on the surfaces, which can be utilized specific ligand-receptor interaction by the free PEG chain end retaining minimized non-specific adsorption of biomolecules as shown in Figure 1. For this objectives, three types of surface modification agents were prepared.(Figure 2)

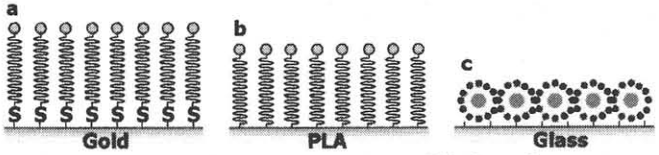

Figure 2. Several types of PEG modified surfaces. a: CHO-PEG-SH on gold; b: CHO-PEG/PLA block on PLA; c: CHO-polymer micelle on glass

Recently, surface plasmon resonance of gold surface was utilized for the analysis of ligand-receptor interaction with extremely high sensitivity. The prepared CHO-PEG-SH can be utilized for modification of this SPR sensor by the conjugation via SH group (Figure 2a). After the modification of SPR chip by acetal-PEG-SH (Mn=10,000), protein adsorption tests were carried out. Figure 3 shows lysozyme adsorption monitored by the SPR. Contrary to the commercial gold and carboxymethyl cellulose (CMC) chips, the PEG tethered chains surface suppressed lysozyme adsorption significantly. After the modification of free aldehyde group by biotin, specific interaction with aviodin protein was observed keeping low non-specific adsorption.

Hydrophobic PEA segment in CHO-PEG/PLA block copolymer was adsorbed on the hydrophobic surface to construct PEG tethered cahin as shown in Figure 2b. This type of tethered chain shows also the same perfoemance with PEG-SH on gold surface and utilized for several types of interfaces such as qualtz, glass, silicon and plastics.

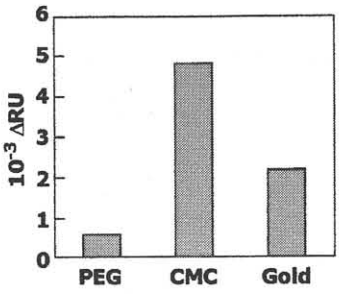

Figure 3. Lysozyme adsorption on the SPR sensor chip

The association number of the CHO-PEG/PLA micelle was estimated ca. 100 in aqueous media. Thus, 100 chains were introduced on the surface via only one covalent linkage on the surface (Figure 2c). Such a micelle modification can be anticipated for construction of dense tethered PEG chains on the surface. After a glass surface was aminated by 3-aminopropylsilaol, CHO-micelle was reacted via a reduced amination reaction. Figure 4 shows AFM picture of the modified surface. The modified surface was covered by the micelle almost completely. The ζ- potential of the surface was measured to be almost zero, indicating again complete shielding of glass surface charge by the micelle layer. The non-specific adsorption of several proteins was also confirmed to suppress in this surface. These types of surface modification agents are promising for high performance biocompatible surface.

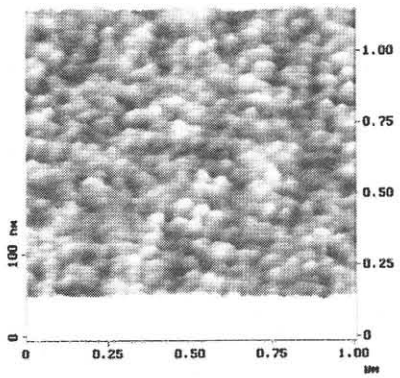

Figure 4. Tapping mode AFM images of CHO-polymer micelle modified glass surface

References

[1] R. Schuster, V. Kirchner, P. Allongue, G. Ertl, *Science* **2000**, 289: 98

[2] R.F. Service, *Science* 1998 October 16; 282: 397

[3] *Polymer Surfaces and Interfaces II*; Feast, W. J.; Munro, S.; Richards, R. W., Eds.; John Wiley & Sons: 1993.

[4] H. Otsuka, Y. Nagasaki, K. Kataoka, *Polyesters of Biomedical and Environmental Importance,* C.Scholz, R.A. Gross, Eds.; ACS Symposium Series 764, Washington, DC, **2000**, pp.311-327