Electrochemically active, anti-biofouling polymer adlayers on indium-tin-oxide electrodes†

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Here we report the synthesis of a novel electrochemically active polymer, preparation of adlayers of the polymer on optically transparent electrodes, and an application of the adlayers to immobilization of engineered cells through a direct covalent coupling reaction.

Recently, a great deal of effort has been made to immobilize various biomolecules on spatially confined positions of a pre-existing electrodes array using electrochemical methods that facilitate the precise and reproducible positioning of biomolecules since control over an individual electrode is possible.[1–9] One of the key components for the patterning is electrochemically active adlayers present on electrode surfaces, through which biomolecules can be immobilized. It is favorable that the electrochemical reactions of the adlayers occur within mild potential ranges under aqueous buffer conditions to retain the native activities of the biomolecules.[4] It is also favorable that an anti-biofouling property is given to the adlayers to prevent nonspecific adsorption of biomolecules which otherwise often generate false-positive signals.[10,11] Although Au electrodes have been widely used for the preparation of such adlayers, they have limited use in biological assays due to their opaque characteristics; in that sense, the use of optically transparent electrodes (OTEs) such as indium-tin-oxide (ITO) may pave the way for potential applications such as cell based assays that rely mainly on optical microscopic observation techniques.[12]

Here we report (i) synthesis of a novel electrochemically active polymer, (ii) preparation of adlayers of the polymer on OTEs, and (iii) an application of the adlayers for immobilization of engineered cells through a direct covalent coupling reaction.

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Very recently, we reported a simple method for the immobilization of biomolecules onto oxide-based substrates based on polymeric self-assembled monolayers of a random copolymer, poly(TMSMA-*r*-PEGMA-*r*-NAS) comprising a trimethoxysilane part for anchoring onto SiO_2-based substrates, a polyethylene glycol part for blocking non-specific protein adsorption (anti-biofouling), and an activated ester for reaction with biomolecules.[13] The electrochemically active polymer was prepared by the modification of the poly(TMSMA-*r*-PEGMA-*r*-NAS). We introduced an electrochemically active *N*-(4-hydroxyphenyl) (NHP) moiety by reacting the copolymer with 4-aminophenol, producing a new polymer, poly(TMSMA-*r*-PEGMA-*r*-NHP) in high yield (Fig. 1a) (see Supporting Information for the synthetic details†). As demonstrated in the case of poly(TMSMA-*r*-PEGMA-*r*-NAS), the electrochemically active copolymer could also form robust adlayers on ITO surfaces through multiple covalent bond formation between the silane groups and the surface hydroxyl groups of the electrode.[13]

Polymeric adlayers (PAs) on ITO electrodes were easily prepared by simply immersing the cleaned substrate in the polymer solution (2.5 wt% in distilled water) for only 1 h. The resulting PAs on the electrodes were rinsed, dried, and baked at 110 °C for 2 min to ensure the covalent bond formation between the trihydroxysilane groups of the polymer and the hydroxyl groups of the electrodes. The pathway for the electrochemical activation of the PAs is illustrated in Fig. 1b, through which thiol containing biomolecules could react with the quinoneimine intermediate that is electrochemically oxidized from an NHP functional moiety.[14,15] Contact angle measurements showed a distinct difference in the hydrophilicity of each surface: $55 \pm 3^\circ$ *versus* $29 \pm 3^\circ$ for a bare ITO surface and the PAs, respectively. The much increased hydrophilicity of the PAs may be due to the presence of hydrophilic PEG layers, indicative of the presence of the PAs on the electrode. X-ray photoelectron spectroscopy (XPS) measurements also revealed that the PAs were well prepared on ITO

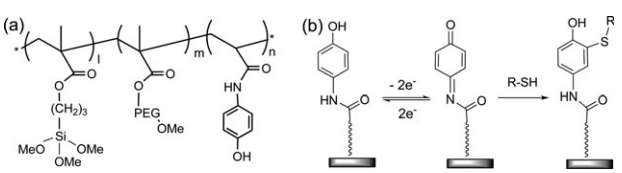

Fig. 1 (a) Chemical structure of an electrochemically active polymer, poly(TMSMA-*r*-PEGMA-*r*-NHP). (b) Electrochemical reaction pathway of the polymer adlayers on an ITO electrode.

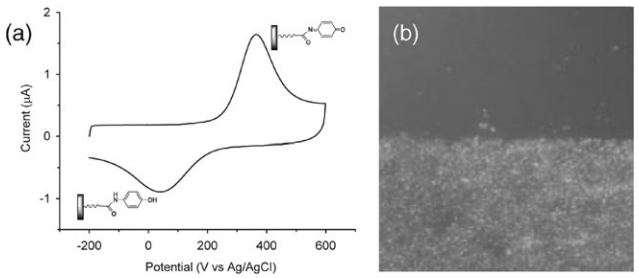

Fig. 2 (a) A cyclic voltammogram for the electrochemical oxidation of the NHP moiety of the PAs on an ITO electrode. Scan rate = 50 mV s^{-1}, working electrode area = 0.08 cm^2. (b) An optical microscopic image of the surfaces of non-oxidized (above) and oxidized (below) PAs taken after reaction with 1,6-hexanedithiol, incubation with gold nanoparticles, and silver enhancement.

electrodes, showing C, O, and N intensities corresponding to the polymer (Fig. S1 and Table S1†).

The ability of the PAs to block nonspecific adsorption of proteins (anti-biofouling property) was examined using a model plasma protein, bovine serum albumin. High resolution N(1s) XPS measurements on the PAs after incubation in the protein solution revealed that ~92% of blocking efficiency was attained when compared to that of a bare ITO surface (Fig. S2†), indicative of an advantage of the present PAs for potential biological applications.

To examine the electrochemical activity of the PAs, cyclic voltammetry (CV) experiments were performed in carbonate buffer solution (0.2 M, pH 9.4). Fig. 2a shows a cyclic voltammogram for the electrochemical oxidation of the NHP moiety in the PAs, in which redox peaks were well-resolved and electrochemically quasi-reversible.[16] The surface coverage of electroactive NHP groups calculated from integration of the charge in the CV without considering surface roughness is $\sim 2.3 \times 10^{-10}$ mol cm^{-2}. The coverage is about one third of the maximum value reported previously for alkylsilane monolayers.[17] The result is reasonable, considering that the polymer has a random triblock structure and the PA has a monolayer thickness of ~1.1 nm that was measured on Si/SiO_2 substrates instead of ITO electrodes by using ellipsometry. The peak currents are linearly proportional to the scan rate, as expected for surface confined species (Fig. S3†). The peak separation increases slightly with scan rate. The increment is ~20 mV with each increase of 100 mV s^{-1}. The decrease in the peak upon continuous scan was not significant, implying that the quinoneimine intermediate as an oxidation product is relatively stable. This characteristic is important in that the intermediate is to react with thiol groups of biomolecules. We next investigated the chemical reactivity of the electrochemically oxidized NHP with thiol groups. The PAs on ITO surfaces before and after electrochemical treatments were immersed in ethanol solution of 1,6-hexanedithiol (30 mM in ethanol) and analyzed by XPS. While a S(2p) peak in XPS was not detected for the untreated surface, the peak was found at ~163 eV for the treated one (Fig. S4†), indicative of successful attachment of the compound. To examine the reactivity further, only a confined area of the PAs-modified ITO surface was subjected to electrochemical oxidation at ~400 mV by partial dipping of the electrode in the electrolyte solution, and then the whole electrode was immersed in ethanolic solution of 1,6-hexanedithiol. The resulting surface was dipped in an aqueous solution of 10 nm gold nanoparticles for 20 min, followed by treatment with a silver enhancement kit. As shown in Fig. 2b, precipitation of metallic silver was exclusively seen in the oxidized PAs area. Gold nanoparticles did not adsorb on the oxidized PAs without pre-treatment with dithiol, which was also confirmed by a silver enhancement test. Taken together, these results clearly indicate that the PAs are switched to a chemically reactive form on demand by electrical stimuli, which subsequently reacts with thiol containing molecules.

To expand the usefulness of the PAs on ITO electrodes, a novel immobilization method for engineered cells was developed. We incorporated thiol functional groups into surfaces of Jurkat lymphocytes (human acute T-cell leukemia) by a metabolic "oligosaccharide engineering" method as reported previously (see Supporting Information for details†).[18] We next carried out immobilization of the engineered cells containing surface thiols onto the PAs. The PAs were constructed on an interdigitated ITO-electrode array (IDIA) for the cell attachment (Fig. 3a). Only one electrode in the IDIA was electrochemically oxidized at about 400 mV for 10 s and the engineered cell suspension was loaded on the IDIA for 2 h, followed by washing with PBS before analysis. Fig. 3b shows an optical microscopic image of cells on an IDIA. The cell densities of two electrodes before and after electrochemical oxidation, respectively, were in extreme contrast with each other; much higher density was observed in the latter.[19] As a control, few non-engineered cells lacking thiols were seen on the electrochemically oxidized PAs (Fig. 3c). These results imply that the cell attachment can be attributed to the covalent coupling between thiol groups of the cell surface and the reactive intermediates of the oxidized PAs, enabling highly specific and efficient immobilization of cells in an electrochemically addressable manner.

In conclusion, we synthesized a new, electrochemically active copolymer and constructed adlayers of the polymer on OTEs for immobilization of biomolecules. Further, we

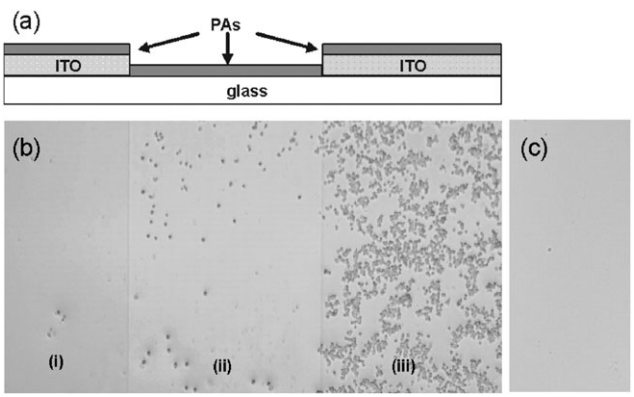

Fig. 3 (a) Schematic side view of PAs-modified IDIA. Transmission microscopic images for (b) spatially selective immobilization of thiol-modified Jurkat cells on an IDIA: (i) non-oxidized PAs on ITO electrode, (ii) PAs on glass, and (iii) oxidized PAs on ITO electrode, and (c) adsorption of native Jurkat cells on the oxidized PAs on an ITO electrode.

demonstrated that spatially selective covalent immobilization of engineered cells could be achieved on IDIA. Potential applications of this material include its integration into microfluidic devices that depend on optical microscopic and electrochemical observation techniques for multiplexed analysis of biomolecules.

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19. The presence of more cells on the glass region (ii) than the ITO-coated region (i) may be caused by the combined effect of two factors. One is the mild washing conditions with light shaking of the substrate in the PBS solution horizontally to remove non-covalently adsorbed cells after the immobilization reaction. The other is the lower height of the region (ii) than the region (i), which may lead cells into the glass ditch during the washing process.