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High performance protein microarrays based on glycidyl methacrylate-modified polyethylene terephthalate plastic substrate

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ABSTRACT

There is a great challenge to immobilize high density of probe molecules for high performance protein microarrays, and this is achieved in this work by using polyethylene terephthalate (PET) plastic substrate onto which glycidyl methacrylate (GMA) photopolymer is grafted under mild conditions to introduce high density of epoxy groups for covalent immobilization of proteins. The poly(GMA)-grafted PET (PGMA-PET) surface was characterized with atomic force microscope (AFM) and attenuated total reflectance Fourier transform infra-red (ATR-FTIR) spectroscopy. For high density of protein immobilization and good quality of microspots, experiments were conducted to optimize the printing buffer, and an optimal buffer was found out to be PBS with 10% glycerol + 0.003% triton X-100. According to the studies of loading capacity and immobilization kinetics, the optimal protein probe concentration and incubation time for the efficient immobilization are 200 $\mu g\,mL^{-1}$ and 8 h, respectively. The performance of the PGMA-PET-based protein microarrays is evaluated with sandwich immunoassay using rat IgG and anti-rat IgG as model proteins, demonstrating a limit of detection (LOD) of 10 $pg\,mL^{-1}$ and a dynamic range of five orders of magnitude which are better than or very comparable with the reported or commercially available immunoassays, while providing a high-throughput approach. The work renders a simple and economic method to manufacture high performance protein microarrays and is expected to have great potentials in broad applications related to clinic diagnosis, drug discovery and proteomic research.

1. Introduction

Bioarrays have demonstrated their increasing impact on clinic diagnostics, drug discovery, and life science research because of their advantages of high-throughput, high reliability, fast analysis and small amount of sample. With the completion of human genome project, exploration and development of various high performance protein arrays have been logically fueled up. Protein microarrays possess the ability to analyze complex biological system and have emerged for numerous important applications including screening of recombinant antibody libraries [1], monitoring of protein–protein interactions [2–4], detection of cytokines [5,6], analysis of protein expression profiling, and identification of protein structure and function [7–9].

Protein microarrays are normally fabricated by delivering various probe proteins onto different substrates such as glass [7–9], silicon [10,11] and gold [12]. Alternatively, organic polymer substrates such as polymethyl methacrylate, polycarbonate and polyurethane are also employed for DNA or protein microarrays [13–16]. Polyethylene terephthalate (PET), one of the most widely used biomaterials in tissue engineering and biomedical devices, is an inexpensive plastic material with desirable physicochemical properties. Up to date, it has not been used to fabricate protein microarrays. In this work, we use it as a potential substrate to fabricate high performance protein arrays by a surface modification.

For microarray construction, one of the most important steps is to efficiently immobilize probe proteins on a substrate. Currently, a number of strategies such as physical adsorption, specific affinity and covalent bonding are available for protein immobilization [17]. Although physical adsorption normally offers the simplest process for the immobilization, it is relatively unstable and uncontrollable. It also unfavorably damages protein activity by protein denaturation and steric hindrance [18]. Alternatively, specific affinity, such as protein A or G with Fc part of an antibody [19] and biotin–avidin/streptavidin interaction [12], are used for site-specific protein immobilization. The approach is easy to retain the native conformation and to control the orientation of immobilized protein. However, it requires conjugation of the probe protein with affinity tag and specifically modified substrate [20]. Covalent coupling provides the strongest attachment of probes onto activated

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substrate [17]. Silanes are often used to functionalize glass slide for covalent protein immobilization [8,21,22], but its immobilization efficiency is low [23]. The steric hindrance between protein and surface from the short spacer provided by silanes also reduces the affinity of probe and the accessibility of analytes.

PET is lack of indigenous reactive functional groups. In tissue engineering and biomedical devices, it is functionalized by saponification, aminolysis and surface reduction for protein immobilization [24]. These methods cannot provide high density of functional groups [24] and require additional crosslinkers such as silanes, glutaraldehyde, aldehyde-dextran and functionalized PEG to covalently immobilize biomolecules [24–27]. Plasma post-polymerization for grafting vinyl compounds onto PET surface is explored for surface modification and biomolecules immobilization [28–32]. However, the plasma approach is slow and expensive [33].

In this work, a simple and efficient approach was investigated to functionalize PET surface by grafting glycidyl methacrylate (GMA) photopolymer for high performance protein microarrays. GMA possesses both acrylate and epoxy groups, of which the former can be employed to photopolymerize GMA in the presence of photoinitiator under UV irradiation, and the latter, can be an anchor for covalent immobilization of proteins. poly(GMA)-grafted PET (PGMA-PET) was prepared and further characterized with atomic force microscope (AFM) and attenuated total reflectance Fourier transform infra-red (ATR-FTIR) spectroscopy. The optimal printing buffer, protein immobilization kinetics and loading capacity were investigated systematically. PGMA-PET-based protein microarrays were fabricated and demonstrated by sandwich immunoassay.

2. Experiments

2.1. Materials and apparatus

1 mm thick PET sheets were purchased from goodfellow (England). Rabbit IgG, biotin-conjugated anti-rabbit IgG, Cy3-conjugated anti-goat IgG, rat IgG, anti-rat IgG, biotin-conjugated anti-rat IgG, hexamethylenediamine, glycidyl methacrylate (GMA), glycerol, triton® X-100 and 0.01 M phosphate buffered saline (PBS, pH 7.4) were received from Sigma–Aldrich. Cy3-conjugated streptavidin was obtained from GE Healthcare. Blocker™ Casein in TBS was purchased from Pierce. Deionized water was produced by a water purification system, Q-Grad®1, Millipore Corporation.

AFM (SPM 3100, Veeco instruments Inc., USA) was used to characterize substrate surface properties. Nicolet 5700 FTIR instrument (Thermo Electron Corporation) was utilized to record the IR spectra. VersArray chip writer™ compact system (Bio-Rad, USA) was applied for microarrays fabrication. ScanArray GX Microarray Scanner (PerkinElmer, USA) was employed for imaging and quantitative analysis.

2.2. Preparation of PGMA-PET slide

The PGMA-PET slides were prepared under mild conditions by following the procedure shown in Scheme 1. For cleaning, the tailored 75 mm × 25 mm PET slides were immersed in 2-propanol for 30 min and then cleaned for 5 min in an ultrasonic water bath, followed by thoroughly rinsing with ethanol and deionized water. The precleaned PET slides were aminated with a method similar to that in the amination of polyester (polymethyl methacrylate, PMMA) [14]. In our preparation, the precleaned PET slides were incubated in 10% hexamethylenediamine for 2 h at room temperature to introduce primary amine to PET ester linkage, and then rinsed three times with deionized water. For the conduction of photografting, GMA monomers were firstly coupled to aminated PET surface via the reaction between epoxy and amino groups to form an acrylate-activated PET surface. Specifically, aminated PET slides were soaked in 5% (v/v) GMA in 50% ethanol solution for 2 h at room temperature, and rinsed thoroughly with ethanol and dried under nitrogen flowing. The acrylate groups enable GMA photopolymer to be covalently attached onto PET surface. The acrylate-activated PET slides were immersed in GMA monomer solution (5% in 50% ethanol, v/v) containing 5 mM sodium persulfate as a photoinitiator, which was completely degassed using nitrogen flow for 30 min. Then, the mixture was exposed to UV (shuttered UV system, 400 W) for 15 min. After the polymerization, PET slides were washed with ethanol and deionized water to remove unpolymerized monomer and uncoupled oligomer or polymer.

2.3. AFM characterization

Pristine, aminated PET and PGMA-PET surfaces were characterized with AFM in a tapping mode. The scan area was 2 μm × 2 μm. All images were acquired in open air.

2.4. ATR-FTIR characterization

The IR spectra were acquired by using a Nicolet 5700 instrument equipped with an attenuated total reflection accessory. The polymerized GMA, pristine PET and PGMA-PET were placed onto the ATR crystal and pressed tightly to obtain good spectra. The examinations were performed by 32 scans with a resolution of 1 (data spacing is 0.482 cm^{-1}).

2.5. Fabrication of protein microarrays

Protein probes were prepared in printing buffer with desired concentrations and then transferred to a 384-well microtiter plate before printing. For printing buffer optimization, 0.01 M PBS containing 10% glycerol and different amount of triton X-100 range from 0.001% to 0.012% were applied as printing buffers. In other experiments, the optimal buffer was employed. For investigation of probe immobilization kinetics and loading capacity, 100 μg mL^{-1} of Cy3-conjugated anti-goat IgG and twofold diluted biotin-conjugated anti-rabbit IgG solutions from 200 μg mL^{-1} to 6.25 μg mL^{-1} were prepared. 20 μL of the protein probe solution was dispensed to each well. The contact printing process was performed by VersArray chipwriter™ (BIO RAD) with telechem printhead and stealth microspotting pins. At 60% humidity, ~0.3 nL of a sample per spot was delivered to PET surface.

Probe immobilization was carried out in a humid chamber with incubation times of 0 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h and 12 h, respectively, for investigation of immobilization kinetics, and overnight for other experiments. Then, the slides were washed three times for 2 min each with PBST (0.01 M PBS with 0.05% Tween 20) to remove unbound probes. All printed array slides were immersed in Blocker™ casein in TBS solution for 1 h not only to quench the unreacted functional groups on substrate surface, but also to form a molecular layer of casein that could reduce the nonspecific binding of other proteins in subsequent steps.

2.6. Imaging and data analysis

Cy3-conjugated streptavidin, which can specifically attach to biotin-conjugated proteins through the strong affinity of biotin and streptavidin, was employed to produce fluorescent signals for detection. After applying Cy3-conjugated streptavidin, slides were thoroughly washed with PBST and deionized water three times,