Construction of High-performance Biosensor Interface through Solvent Controlled Self-assembly of PEG grafted Polymer

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ABSTRACT

Self-Assembled Monolayers (SAMs) are conventionally exploited for modification of the biosensor surface due to its easiness of formation as well as readily tunable properties. However, the lack of 3-D nature and robustness has limited its application. We develop a Polymeric Monolayer (PM) - based on a water-soluble PEG-grafted polymer - that retains the advantages of SAMs and at the same time bears the characteristic of robustness and 3-D. A water-based PM with methoxyl-functionalized Poly-(Ethylene Glycol) PEG exhibits extremely low nonspecific protein adsorption; while a water-based PM with carboxyl-functionalized PEG shows a high amount of antibody binding. Furthermore, the specific nature of our PM allows easy patterning through a combination of lift-off and self-assembly, which is highly attractive in the array-based biosensors. We believe that the solvent controlled self-assembly of our PM offers a simple and highly adaptable way to construct a high-performance biosensor interface.

Keywords: biosensor, interface, poly(ethylene glycol), protein adsorption, copolymer

1 INTRODUCTION

Biosensor has gained importance in the pharmaceutical and medical applications [1]. The properties of the interface layer in the biosensor plays a key role in determining its final performance, such as stability, reproducibility, and sensitivity [2]. Self-assembled Monolayers (SAMs) of alkane thiols are conventionally used interface layer in biosensors. However, an important limitation of the alkane thiols system lies in the relative poor stability of the chemisorbed alkanethiolates [3]. In addition, the intrinsic 2-D nature of the alkane thiol monolayers limits its protein immobilization capacity in comparison with the 3-D matrix [2]. The stability of Polymeric Monolayers (PM), which is formed from a kind of polymers with multiple sulfide or disulfide groups grafted onto a polymer backbone, is significantly improved compared to monomeric alkane thiol monolayers [4]. It is believed that the use of multisite attachment via polysulfides (or polydisulfides) can minimize the loss of functionality due to adlayer degradation, consequently improving the stability of the adlayer. We prepared a functional Poly(ethylene Glycol) PEG derived polysiloxane copolymer, where both functional PEG and alkane disulfide chains are grafted onto a polysiloxane backbone (Figure 1).

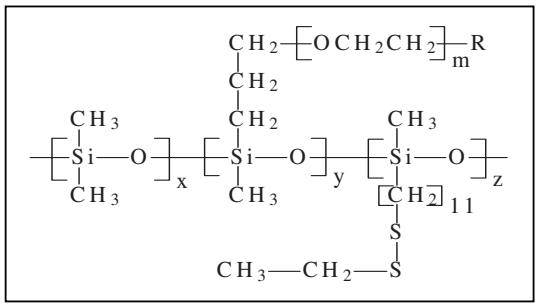

Figure 1: Schematic chemical structure of the functional PEG derived polysiloxane copolymer

The copolymer can chemisorb onto gold to form a PM thorough the grafted multiple disulfide groups. The PEG chains presenting in PM is either functionalized with methoxyl group or carboxyl groups. The grafted PEG chains are expected to provide several advantages: firstly, the nonspecific protein adsorption will be minimized due to the well-know protein resistant ability of the PEG chains [5]; secondly, the immobilization capacity of protein (e.g. antibody) will be enhanced since the relative flexibility of the PEG chains resulting in a 3-D like characteristic [6]. The organization of the resulting PM is controlled by the solvent from which the PM is deposited. Under optimal organization, a methoxyl-functionalized PEG derived PM showed excellent resistance to nonspecific protein adsorption; while a carboxyl-tailed PEG derived PM exhibited a high amount of antibody binding, consequently, a SPR sensor modified by the PM can achieve a detection limit of <1ng antigen. In addition, the patterning of PM shows potential for array-based biosensor applications.

2 MATERIALS & METHODS

2.1 Materials

The functional PEG derived polysiloxane copolymers were prepared as described in [7]. The methoxyl-functional PEG derived polysiloxane copolymer has compositions as following: the Molecular Weight (Mw) of the PEG chain is 1100, the graft ratio (in comparison to the number of the siloxane units in the polysiloxane backbone) of PEG chains is around 74%, the grafted alkane disulfides contains 13 carbons, and the graft ratio of the alkane disulfides is around 15%. The carboxyl-functionalized PEG derived polysiloxane copolymer has similar compositions except that the graft ratio of the PEG chains is 43%.

2.2 PM Preparation and Characterization

The PM was prepared by immersing the clean gold substrates in the 0.5mM solution (water or toluene) of the functional PEG derived polysiloxane copolymers for 6hr. Then the substrates were rinsed by water and ethanol, and dried by N_2.

The surface properties were characterized by the static water contact angle measurement and angel-resolved X-ray Photoelectron Spectroscopy (XPS).

2.3 Nonspecific protein adsorption test

The nonspecific protein adsorption test was performed on a commercial SPR instrument, Biacore2000. The PBS buffer was flowed over the PM modified sensor surface to establish a baseline signal, then 1mg/ml protein solution was allowed to contact the sensor surface for one hour, afterwards, PBS buffer was injected several times to remove any loosely bound proteins. The SPR signal after PBS buffer rinsing was subtracted by the baseline signal, which resulting in a value representing the amount of the adsorbed proteins. In Biacore2000, 10RU signal change corresponds to 1ng/cm^2 protein adsorption.

2.4 Antibody immobilization and antigen recognition

The SPR (Biacore2000) sensor surface modified by carboxyl-functionalized PEG derived PMs were activated by carbodiimide chemistry to covalently bind to the amino groups of antibodies. After the antibody immobilization, the surface was incubated with ethanolamine to block the rest un-reacted carboxyl groups. Then different concentration (from 1ng/ml to 10μg/ml) of antigen was flowed over the sensor surface to check the antigen recognition ability. Human Transferrin (HT) and a Monoclonal Antibody (MAb) against HT were used as model system. During the recognition test, sensor surface immobilized with a similar MAb but with no affinity to HT was used as control surface. The SPR signal of HT recognition on anti-HT immobilized surface was subtracted by the signal of HT interaction on the control surface, which gives a reliable estimation of specific HT recognition on the sensor surface. Meanwhile, 10μg/ml Human IgG and 100μg/ml were used as a control of nonspecific adsorption.

2.5 Cells culture and pattering

3T3 mouse embryonic fibroblasts (ATCC CCL-92) were obtained from the American Tissue Type Culture Collection (Bethesda, MD). Cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10 % fetal calf serum, 3.8 mM L-glutamine, 0.9 % (v/v) non-essential amino acids, 85 IU/ml penicillin, 85 μg/ml streptomycin and 20 mM HEPES (pH 7.4). Cells were routinely passaged twice a week at a density of 2 x 10^5 cells per 75 cm^2 flask. For experiments cells were seeded in six-well clusters (Costar, MA) at a density of approximately 8 × 10^3 cells·cm^{-2}. The gold substrates coated with patterning of PM were fixed in the wells by autoclaved silicon grease. Cell growth was monitored and cells were photographed using an inverted microscope (Zeiss) 1 day after seeding.

3 RESULTS AND DISCUSSION

3.1 Solvent effect on surface properties of the PM

As shown in table 1, for both methoxyl-functionalized PEG derived PM and carboxyl-functionalized PEG derived PM, the solvent from which the PMs were deposited has great impact on the surface properties of the PMs.

	static water contact angle		*C-O content (%)			
	water-based	toluene-based	water-based		toluene-based	
methoxyl-functionalized PEG derived PM	31˚±3˚	56˚±2˚	5˚	80˚	5˚	80˚
			63±1	73±1	62±1	55±1
carboxyl-functionalized PEG derived PM	26˚±3˚	42˚±2˚	Not test		Not test	

Table 1: the static water contact angle value and C-O content of the functional PEG derived PM deposited from water and toluene based solutions. (* the C-O content was calculated from the content of C element using the C-O percentage of the overall C_{1s} peak. Only C, Si, S elements were taken into account in composition calculation.)

The PMs deposited from water-based solutions have lower static water contact angle value than the PMs deposited from corresponding toluene solutions. Both

water-based PMs with methoxyl-functionalized PEG and carboxyl-functionalized PEG show hydrophilic properties. In contrast, the corresponding toluene-based PMs exhibit relative hydrophobic properties. Since the PEG units in our copolymers has hydrophilic properties while the alkane disulfides and polysiloxane backbone are mainly hydrophobic, the static water contact angle values suggest that there are more PEG units enriched at the outmost surface for water-based PMs compared to toluene-based PMs.

The angle-resolved XPS data corroborate the results from the water contact angle measurement. For water-based PM with methoxyl-functionalized PEG, the content of ether carbon, i.e. C-O, which is the characteristic of PEG units, decreases with decreasing incident XPS angle (from 80° to 5°), i.e. increasing sampling depth (table 1). It indicates that the PEG chains are enriched at the outmost surface of the PMs. In contrast, the C-O content in the corresponding toluene-base PM increased with increasing sampling depth (table 1). It indicates that quite a lot PEG chains are buried under the polysiloxane backbones in the toluene-base PMs.

3.2 Nonspecific protein adsorption

The nonspecific protein adsorption test was performed on SPR sensor surface modified by methoxyl-functionalized PEG derived PMs. Human Fibrinogen (FB) was used as model system due to its strong surface adhesion properties. The amount of adsorbed FB on the water-based PM is much less than that on the corresponding toluene-based PM (Figure 2). The water-based PM shows excellent resistance to the nonspecific protein adsorption, less than 0.1ng/ml as converted from SPR signal, which is comparable with the best protein resistance surface reported by now [8].

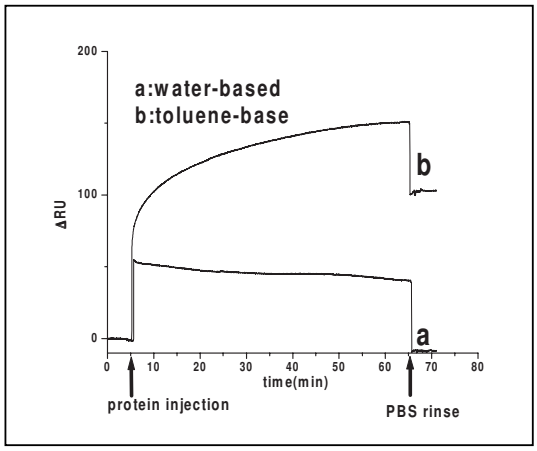

Figure 2: SPR sensogram for FB adsorption on methoxyl-functionalized PMs: curve a: water-based; curve b: toluene-based PM

The nonspecific protein adsorption test data is in consistence with the surface characterization results. As it is well-known that the PEG chains are efficient in resisting nonspecific protein adsorption [5] and there are more PEG enriched at the outmost surface on water-based PM, the water-based PM exhibits better protein resistance properties.

3.3 PM as interface for Immunosensor

The potential of using the carboxyl-functionalized PEG derived PMs for immunosensor application was explored. Human Transferrin (HT) and Monoclonal Antibody (MAb) against HT were chosen as models system. The interaction between the HT and immobilized anti-HT was monitored by the SPR signal.

As expected, the water-based PM with carboxyl-functionalized PEG can immobilize two times higher amount of anti-HT (4000RU) than the corresponding toluene-based PM (1700RU). It is because more PEG and carboxyl groups are exposed at the outmost surface of the water-based PM than at the corresponding toluene-based PM.

The consequent HT recognition was performed on water-based PM immobilized with anti-HT. As summarized in figure 3, the 1ng/ml HT can be readily detected while there is almost no detectable nonspecific Human IgG (10μg/ml and 100μg/ml) adsorption.

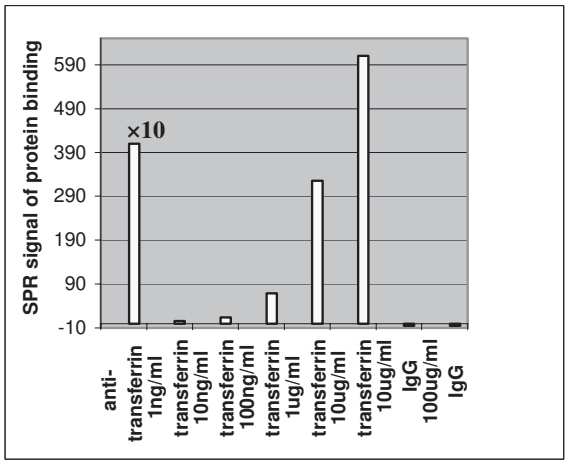

Figure 3: The recognition of transferrin (concentration varied from 1ng/ml to 10μg/ml) on the anti-transferrin that was immobilized on the water based PM with carboxyl-functional PEG. Human IgG (100μg/ml and 10μg/ml) were used as a control of nonspecific adsorption.

The model antigen/antibody test suggests that water-based PM with carboxyl-functionalized PEG is a very promising interface layer for construction of biosensors.

3.4 Patterning of PM

As demonstrated above, the water-based PM have the advantage of the corresponding toluene-base PM for the surface modification of biosensor, at the same time the nature of the water-base PM make it feasible to obtain a patterning of PM through the combination of self-assembly and lift-off [9]. In the process, a photoresist was first spin-coated onto a clean gold substrate. Next, the pattern was transferred into the photoresist from a photolithography mask by UV-illumination and development. After these steps, part of the gold substrate was covered and protected by photoresist while other areas are exposed. Subsequently, this gold substrate was immersed into a water based solution of methoxyl-functionalized PEG derived polysiloxane copolymer for 6hrs. The copolymer chemisorbed onto the exposed gold areas while it physically adsorbed on the photoresist-protected areas. Afterwards, the substrate was taken out of the copolymer solution and rinsed with an organic solvent. This organic solvent removed the photoresist together with the physically adsorbed copolymer, while the chemisorbed copolymer remained on the gold surface. As a result, a gold substrate with a patterned copolymer structure on it was obtained. If an organic solvent based solution, such as toluene solution of the copolymer, would have been used, the photoresist would have dissolved, and the pattern would have been destroyed, during the deposition of the PM.

Due to the protein resistance property of the PM with methoxyl-functionalized PEG, the resulted patterning of this PM can control the organization of the protein as well as cells. Figure 4 shows 3T3 cells growing on the gold substrate modified with patterning of methoxyl-functionalized PEG derived PM. It is clear that the cells only grew in the area void of PM. Consequently, a pattern of living cells was achieved.

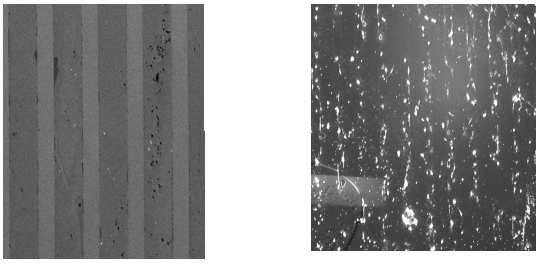

Figure 4: The polymer pattern (left image) formed by combination of lift-off and self-assembly (the dark strips are PEG grafted polymer); the 3T3 cells growing on the polymer pattern (right image) (the bright spots are cells bodies)

If the gold substrates were selectively modified by both carboxyl-functionalized PEG derived PM and methoxyl-functionalized PEG derived PM. One can define the areas on the substrates either with protein (or cells) repellent properties or with protein (or cells) adhesion properties. Therefore our method of pattering the PM provides an easy way to construct array-based biosensors [10].

4 CONCLUSION

We have demonstrated a kind of functional PEG derived PMs. The organization and consequently the surface properties of the PM are controlled by the solvent from with the PM is deposited. A water-based PM has more PEG units enriched at the outmost surface in comparison with the corresponding toluene-based PM. For biosensor application, the water-based PMs have advantage of the toluene-base PMs in view of the resulting surface properties.

We have showed two kinds of potential applications of the water-based PM. A water-based PM with methoxyl-functionalized PEG exhibits extremely low nonspecific protein adsorption (<0.1ng/cm^2). A water-based PM with carboxyl-functionalized PEG is able to efficiently immobilize antibody and consequently achieve a detection limit of <1ng/ml antigen.

We also explored the potential of such PMs in construction of array-based biosensors, as a patterning of PM can be readily achieved through combination of self-assembly and lift-off.

We believe this kind of functional PEG derived PM together with its solvent controllable surface properties provide a simple, robust and efficiently way for construction of interface in biosensor application.

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