A nanofibrous membrane with tunable surface chemistry: preparation and application in protein microarrays

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A nanofibrous membrane fabricated by electrospinning (ES) technology has found a pyramid of promising applications; to satisfy the demand of various applications, nanofibrous membranes with tunable surface chemistry are needed, and thus effective surface modification strategies for functionalizing nanofibers are highly desired. To this end, we herein report a general and convenient method to functionalize ES nanofibers. We have developed a technology that produces ES nanofibers with initiators covalently anchored on the fiber surface, which we term as iES. Subsequently, the iES nanofibers are used as a general scaffold for surface modification *via* surface initiated polymerization (SIP). The tunable surface chemistry, determined by application needs, was realized by the choice of specific monomers for SIP, which resulted in the desired functional polymer surface. The nanofibrous membrane modified *via* this method was demonstrated as the substrate for protein microarrays, showing excellent performance. Given the generality and simplicity of this method, we believe that the combination of iES and SIP will be a robust platform for extending the application of ES nanofibers.

Introduction

We present a facile and general method for the surface modification of nanofibers to produce a nanofibrous membrane with tunable surface chemistry by combining two technologies, namely initiator integrated electrospinning (iES) and surface initiated polymerization (SIP). Electrospinning (ES) is a well-developed technology that processes polymeric materials into nanofibers.[1–5] Electrospun mats have shown excellent performance for various applications such as scaffolds for tissue engineering,[6,7] supports for catalysts,[8,9] molecular sensors,[10,11] and substrates for microfluidics and protein microarrays.[12–14] For these applications, the surface properties of the electrospun mats play important roles. For example, for immunoassay substrates, the surface should be elaborately tailored to enhance the adsorption of target proteins, yet minimize the nonspecific protein adsorption.[14] However, it's difficult to have one material to meet all application needs. Moreover, not all materials with desired functionality can be easily fabricated into continuous fibers *via* ES. Therefore, a general surface modification strategy for ES nanofibers might be a good choice because it replaces the demand of time-consuming ES of different new materials with easily-achieved surface modification of nanofibers, especially when cost-effective electrospun compatible polymers are used as general scaffolds.

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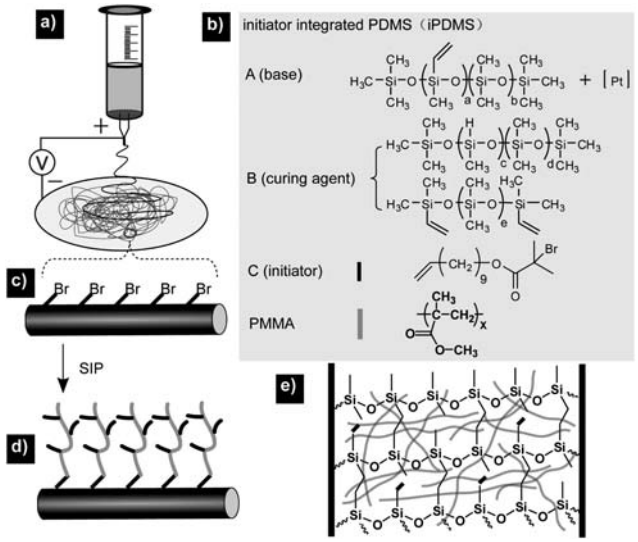
Different strategies were developed for the modification of electrospun nanofibers, mainly including physisorption[15] and chemical coupling.[16–19] SIP (*i.e.*, the "grafting from" strategy) is a powerful tool for modifying various surfaces, which could control the functional groups, density and thickness of polymer brushes with molecular level precision.[20,21] To achieve SIP, initiators must first be efficiently immobilized on to the surface of nanofibers. Fu *et al.*[19] developed a method that first used atom transfer radical polymerization (ATRP) to synthesize initiator-anchored polymers, followed by electrospinning the anchored polymers into nanofibers, thus immobilizing the initiators on the surface of the nanofibers. This method was time consuming because it started from monomers. We have developed a simple method to immobilize vinyl-terminated initiators in bulk poly (dimethylsiloxane) (*i.e.*, initiator integrated PDMS, iPDMS), in which initiators were covalently linked in the PDMS network.[22,23] In order to employ this method in the ES system, we first needed to fabricate PDMS nanofibers. However PDMS is difficult to electrospin due to the small molecular weight of the PDMS prepolymer, resulting in insufficient chain entanglements for ES. Alternatively, we used poly(methyl methacrylate) (PMMA) as the carrier polymer to aid the ES process, resulting in PDMS/PMMA nanofibers.[14] Through the combination of iPDMS and PDMS/PMMA nanofibers, we realized herein the fabrication of iPDMS/PMMA nanofibers, which were further subjected to SIP, resulting in core–shell structures. The iPDMS/PMMA nanofiber core can be considered as a general scaffold, and the polymer chain shell formed after SIP can be rationally designed to cater for the desired applications by choosing specific monomers. This system has three advantages: universality, simplicity and applicability.

The protocol includes the following four steps (Scheme 1): (i) prepare the blend solution of PMMA and iPDMS (molecular formula shown in Scheme 1b); (ii) ES iPDMS/PMMA into



Scheme 1 Illustration of the protocol for surface modification of iES nanofibers. (a) ES setup. (b) Molecular formula of PDMS base, curing agent, initiator and PMMA. (c) After iES and curing, initiators are immobilized on the surface of nanofibers. (d) Polymer brushes are grafted on the surface of nanofibers *via* SIP. (e) A scheme of the 3D network of iES nanofibers.

nanofibers (Scheme 1a); (iii) heat iPDMS/PMMA at the desired elevated temperature to cure the thermosetting PDMS and immobilize initiators in the PDMS 3D network (Scheme 1c and 1e); (iv) finally functionalize nanofibers *via* SIP with the target monomers (Scheme 1d).

Experimental section

Materials

Poly(methyl methacrylate) (PMMA, Mw = 350000), oligo (ethylene glycol) methacrylate (OEGMA, 98%), 1*H*,1*H*,2*H*,2*H*-perfluorodecyl methacrylate (FMA, 99%), and hydroxyethyl methacrylate (HEMA, 97%), were purchased from Aldrich and used as received without any treatment. Poly(dimethylsiloxane) (PDMS, Sylgard 184) was obtained from Dow Corning and consisted of liquid components A (a mixture of catalyst Pt and prepolymer dimethylsiloxane with vinyl groups) and curing agent B (prepolymer dimethylsiloxane with vinyl groups and Si–H groups). The vinyl-terminated initiator (v-initiator, undec-10-enyl 2-bromo-2-methylpropanoate) was purchased from HZDW (99%, Hangzhou, China). Dimethylformamide (DMF) and tetrahydrofuran (THF) were from Sinopharm Chemical Reagent Co, Ltd.

Preparation of iPDMS/PMMA solution

A measured amount of PMMA was dissolved in DMF–THF (1 : 1, by weight) at 50 °C to yield a transparent solution. When the PMMA solution was cooled to room temperature, a specified amount of PDMS (Sylard 184 base and curing agent) together with vinyl-terminated initiator was added into the PMMA solution. After an adequate mixing a milky white, semi-transparent solution was obtained.

Electrospinning

Electrospinning equipment was built on a DC high-voltage generator (Spellman SL150) that can produce a voltage up to 30 kV. We loaded the iPDMS/PMMA solution into a 5 mL syringe and used a syringe pump (Longerpump™ LSP02-1B) to feed the solution at a constant speed. A flat needle was fixed at the bottom of the syringe and connected with the anode of the high-voltage generator. A sheet of aluminium foil was placed under the needle, which was connected with the cathode, and also acted as the collector. The electrospun mats were cured at 70 °C for 2 h.

SIP of iES nanofibers

We cut the iES mats together with aluminium foil into small pieces, then the iES mats were peeled off from the foil and adhered to a double face adhesive tape with the other side on a glass slide. The reaction mixture for SIP was prepared by the thorough mixture of two parts. Part 1 was a transparent, pale-blue solution, which was prepared by adding a specified amount of $CuCl_2$ (5.4 mg, 0.04 mmol), 2,2′-bipyridine (Bipy, 12.5 mg, 0.08 mmol, *i.e.*, 1 : 2 mole ratio), and a fixed amount of monomer to 5 mL of Milli-Q water. Part 2 was a colorless solution, which was prepared by adding a specified amount of ascorbic acid (AscA, 7.0 mg, 0.04 mmol) to 5 mL of Milli-Q water. After both solutions were deoxygenated separately, the two parts were mixed together under argon. The mixture was further deoxygenated, and the resulting mixture turned red due to the reduction of the Cu(II)/Bipy complex to Cu(I)/Bipy. The resulting mixture had a mole ratio of monomer/$CuCl_2$/Bipy/AscA = 50/1/2/1, with a feed [$CuCl_2$] of 0.04 mM. This mixture was then transferred to a closed box with an argon atmosphere. SIP was initiated when iES mats were immersed in the mixture at 25 °C. The polymerization was stopped when iES mats were taken out from the solution and exposed to oxygen. Samples were thoroughly rinsed and cleaned with Milli-Q water and dried at 35 °C.

Scanning electron microscopy (SEM)

A Quanta 400 FEG SEM (FEI) was used to observe the morphology of the electrospun mats. Before observation, all samples were sputter-coated with a gold coating, about 10 nm thick, to decrease the charge effect.

Transmission electron microscopy (TEM)

We dispersed the electrospun mats in ethanol with ultrasonic treatment for 5 min and dropped the dispersion solution on the copper grids. After the ethanol evaporated, nanofibers were deposited on the surface of the copper grids. We employed a TEM (Tecnai G2 F20 S-Twin 200 kV) to observe the samples.

X-Ray photoelectron spectroscopy (XPS)

We used XPS (AXIS Ultra by Kratos Analytical, UK) to determine the surface composition of iES nanofibers. Monochromatic Al Kα X-rays (1486.7 eV) were employed. The X-ray source, with a 2 mm nominal X-ray spot size, was operated at 15 kV, 8.9 mA for both survey and high resolution spectra. Survey spectra, with 0 to 1200 eV binding energies (BE), were recorded at 160 eV pass energy with an energy step of 1.0 eV and

a dwell time of 100 ms. High-resolution spectra were recorded at 20 eV pass energy with an energy step of 0.1 eV and a dwell time of 1.2 s, with a typical average of 12 scans. The operating pressure of the spectrometer was typically $\sim 10^{-9}$ mbar. For quantitative XPS measurements, a survey scan was first taken at an angle of 90°, defined as the angle between the collection axis of photoelectron analyzer and sample plane. All data were collected and analyzed using software provided by the manufacturer.

Differential scanning calorimetry (DSC)

The differential thermal analyzer (SII NanoTechnology, DSC 6220) was operated in nitrogen gas from room temperature to 180 °C at a heating rate of 5 °C min^{-1}.

Protein microarray

We choose a standard sandwich protocol for the protein microarrays. First, an automatic microarray arrayer (BIODOT AD1500) was used to spot antigen solutions (Goat IgG, 10 nL/dot) on the chip in 4 × 4 arrays at room temperature and 60% constant humidity. After overnight immobilization, the microarrays were incubated with antibody solutions (Rabbit *anti*-Goat IgG, abbreviated as R *anti*-G IgG thereafter) for 30 min, and then washed with tris buffered saline Tween20 (TBST). Third, microarrays were incubated with detection antibody solutions (TRITC conjugated G *anti*-R IgG) for another 30 min, and then washed again with TBST. Last, after drying the microarray chip with a N_2 air flow, we read the fluorescent signals with a microarray scanner (CapitalBio LuxScanTM10K).

Results and discussion

In comparison with the ES of PDMS/PMMA nanofibers, iES used iPDMS instead of PDMS. Because iPDMS only added a small amount of initiator to the blend of PDMS base and curing agent, ES of iPDMS/PMMA should be similar to that of PDMS/PMMA. Our previous demonstration of fabricating PDMS/PMMA nanofibers proved three important prerequisites for iES:[14] (i) continuous PDMS/PMMA nanofibers with uniform diameter were successfully fabricated; (ii) the composition of the surface of PDMS/PMMA nanofibers was similar to that of the feed bulk solution; (iii) PDMS could cure in the blend of PDMS and PMMA. The optimized experimental parameters for the ES of PDMS/PMMA nanofibers are as follows, which are also applicable to the ES of iPDMS/PMMA nanofibers: DMF–THF = 1 : 1, PMMA concentration 6%∼10%, PDMS/PMMA 2 : 1, working distance of 13 cm and a voltage of 26 kV. Scheme 1 shows the process of iES technology. We fixed the ratio of PDMS base A/curing agent B = 10 : 2, and altered the amount of initiator C (*e.g.*, A/B/C = 10 : 2 : 0.1) to adjust the initiator content in iPDMS, which will influence SIP. When iES nanofibers were heated at evaluated temperature (*i.e.*, 70 °C), the vinyl groups in the PDMS base and the hydrosilane hydrogens in the curing agent would undergo a hydrosilylation reaction in the presence of the Pt catalyst to form a 3D cross-linked network; at the same time, the initiator would also covalently anchor in the cross-linked PDMS network *via* a hydrosilylation reaction (Scheme 1c and 1e).[22]

We hypothesized that PMMA macromolecular chains intercalated in PDMS 3D cross-linked networks as shown in Scheme 1e, and as a result, the PMMA chains were seriously confined by the PDMS network. DSC results verified such an assumption. We used DSC to measure the glass transition behavior of PDMS, PMMA and iES nanofibers at the temperature range 30∼180 °C. Not surprisingly, cross-linked PDMS had no thermal response in this temperature range and PMMA showed a glass transition at about 110 °C. It is worth noting that cured iES nanofibers had no thermal response, similar to PDMS (Fig. 1). The DSC results indicated that the PDMS 3D network restricted the mobility of PMMA chains, and so cured iES nanofibers had no thermal response. The results also supported another two assumptions: (i) phase separation did not occur between PDMS and PMMA, otherwise the PMMA in iES nanofibers would have shown a glass transition response; (ii) PDMS was indeed cured in the blend of iPDMS/PMMA, otherwise there was no network to restrict the mobility of the PMMA chains. The formation of a 3D network was also supported by XPS analysis: elemental Br, from the initiator, was detected on the surface of iES nanofibers (insert in Fig. 2). The fact that the value of atom % for Br was lower than the calculated value was attributed to the photobleaching effect of XPS on Br and the loss of initiators during the curing process.

Next we prepared a nanofibrous membrane with tunable surface chemistry by functionalizing iES nanofibers with different monomers *via* SIP. OEGMA, FMA and HEMA were used as model monomers. The detailed process of SIP was described in the experimental section. XPS, SEM and TEM results verified the success of SIP. Since FMA has a diagnostic F peak in the XPS measurement, we used it to demonstrate the change of composition of iES nanofibers before and after SIP, which resulted in a coating of poly(FMA) chains around the iES nanofibers. Compared with iES nanofibers, poly(FMA) modified iES nanofibers showed a unique F 1s peak at 693 eV, which proved that poly(FMA) presented at the surface of iES nanofibers (Fig. 2). Moreover, Table 1 shows that, with the increase of initiator C from 0.001 to 0.1 in iPDMS, the concentration of F increased from 5.46 to 43.95 atom %, and Si decreased from 16.14 to 3.08%, respectively, indicating that the density of

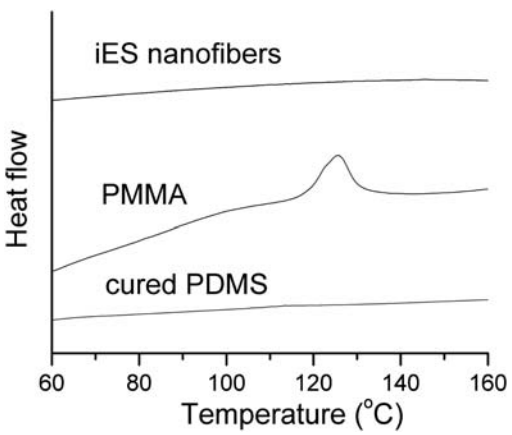


Fig. 1 The DSC measurements for the cured PDMS, PMMA and the cured iES nanofibers.

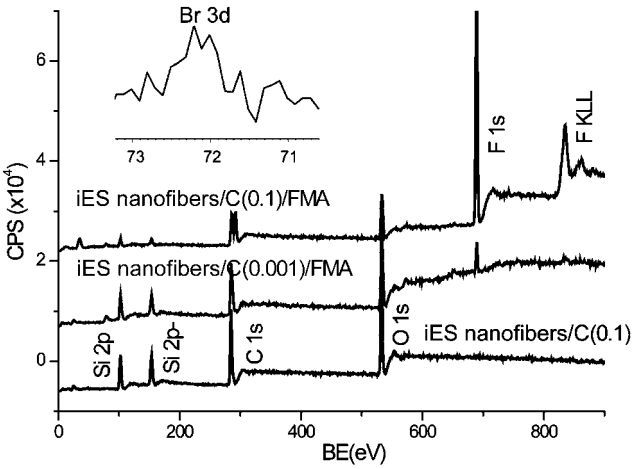


Fig. 2 XPS analysis of iES nanofibers and poly(FMA) modified iES nanofibers. Insert is the Br signal of the iES nanofibers. FMA/$CuCl_2$/Bipy/AscA = 50 : 1 : 2 : 1, reaction time 1.5 h.

Table 1 XPS analysis of iES nanofibers and FMA modified iES nanofibers

	Atom % [a]			
	C	O	Si	F
iES nanofibers/C(0.1)	52.52	28.16	19.33	0.00
iES nanofibers/C(0.001)/FMA	51.59	26.80	16.14	5.46
iES nanofibers/C(0.1)/FMA	42.97	10.00	3.08	43.95

[a] Atom % was based on the survey scan of Si 2p, C 1s O 1s and F 1s.

poly(FMA) chains covering the surface of iES nanofibers increased with the increment of initiator. The XPS measurement of OEGMA and HEMA modified iES nanofibers also proved that poly(OEGMA) and poly(HEMA) brushes were grafted on the surface of iES nanofibers in that the shape of the C1s spectra exhibited some change (data not shown) due to the alteration of the neighbor groups.

SEM and TEM further confirmed the success of SIP by clearly showing the morphology change of iES nanofibers after SIP. Similar to PDMS/PMMA nanofibers, iES nanofibers were smooth and continuous, and with uniform diameters (Fig. 3c). After SIP with different monomers, iES nanofibers showed apparent changes in morphology (Fig. 3d–f): the diameter of iES nanofibers increased; iES nanofibers, especially at the intersection of every two nanofibers, were fused together by the grafted polymer chains. TEM verified that grafted polymer chains formed an SIP shell on the surface of the iES fiber core (Fig. 4). TEM results also indicated that this method should be an efficient solution to prepare core–shell structures. To confirm that SIP could be initiated only from the surface of iES nanofibers, and exclude the possibility that the polymer chains were the product of self-polymerization of the monomers, we carried out two control experiments. The first was to use PMMA nanofibers instead of iES nanofibers. After SIP, the morphology of PMMA nanofibers did not change (Fig. 3a). In another experiment, we conducted the same SIP procedure on PDMS/PMMA nanofibers (without initiators): no polymer chains were observed on the surface of PDMS/PMMA nanofibers (Fig. 3b). XPS, SEM and TEM results demonstrated that polymer chains were grafted on iES nanofibers, and formed core–shell structures.

The most critical parameters that influenced the synthesis of polymer chains were initiator ratio and the SIP time. The initiator ratio in iPDMS determined the initiator density of iES nanofibers, and thus affected the thickness of the polymer chains. SIP time had a similar influence on the diameter of iES nanofibers. Therefore, one can control the thickness of the polymer chains by tailoring the initiator ratio and SIP time. However, if the SIP time was excessively long, such as 180 min, all the nanofibers were fused together to form an integrated membrane (Fig. 5b), as compared with the individual nanofibers for a relatively short SIP time (Fig. 5a).

To semi-quantitatively determine the amount of grafted polymer brushes, we calculated the graft ratio and measured the thickness of the grafted poly(OEGMA). After SIP for 1 h and rinsing thoroughly, for example, the weight of 13.2 mg iES films increased to 14.8 mg. The extra 1.6 mg mass was considered to be the weight of grafted poly(OEGMA). So the graft ratio was about 1.6/13.2 = 12%. Furthermore, we combined the SEM observation and semi-quantitative calculation to obtain the thickness of grafted poly(OEGMA), being about 39 ± 5 nm, which agreed well with the TEM results.

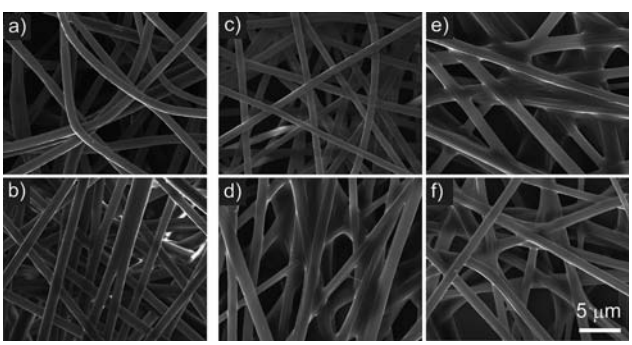


Fig. 3 SEM images of iES nanofibers: (a) PMMA nanofibers with SIP of OEGMA; (b) PDMS/PMMA nanofibers with SIP of OEGMA; (c) iES nanofibers; (d–f) iES nanofibers with SIP of HEMA, OEGMA, FMA, respectively. PMMA 10%, A/B/C = 10 : 2 : 0.01, monomer/$CuCl_2$/Bipy/AscA = 50 : 1 : 2 : 1, reaction time 1.5 h.

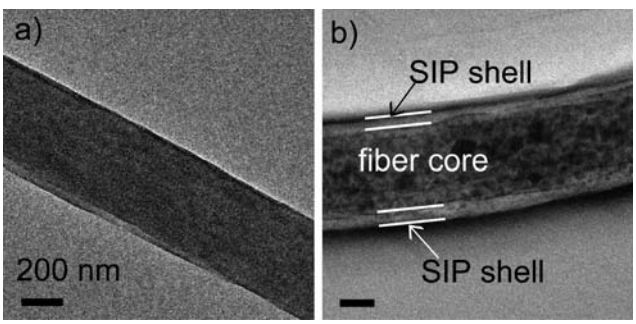


Fig. 4 TEM images of (a) iES nanofibers, (b) Poly(OEGMA) modified iES nanofibers. PMMA 6%, A/B/C = 10 : 2 : 0.01, OEGMA/$CuCl_2$/Bipy/AscA = 50 : 1 : 2 : 1, reaction time 1.5 h. Both (a) and (b) share the same scale bar. The core has a diameter about 430 nm, and the shell thickness was about 65 nm.

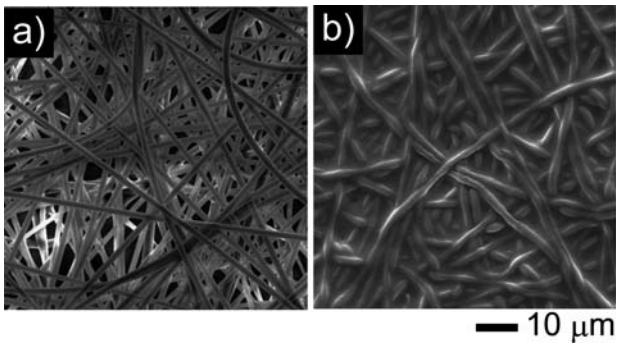


Fig. 5 SEM images of iES nanofibers with SIP of OEGMA for different reaction times: (a) 15 min; (b) 180 min. PMMA 10%, A/B/C = 10 : 2 : 0.001, OEGMA/$CuCl_2$/Bipy/AscA = 50 : 1 : 2 : 1.

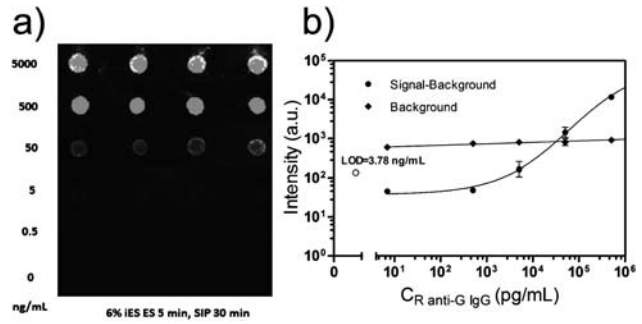


Fig. 6 A poly(OEGMA) modified iES nanofibrous membrane as a solid substrate for protein microarrays. (a) The fluorescent image of protein microarrays. (b) Dose–response curves and LOD for R *anti*-G IgG. The Y axis was the average fluorescence intensity in spots (ball) and in background (diamond), and the X axis was the concentration of R *anti*-G IgG. Error bars represent the standard deviation.

Because of the nonuniformity of the iES nanofibrous membrane and the complexity of SIP reactions, it was not easy to precisely control the reaction dynamics as well as the thickness of grafted polymer chains. Such drawbacks are inevitable and acceptable for surface modifications at the nanoscale. Since the final and most important goal of this research is improving the functionality and applicability of the electrospun nanofibers, we believe that the degree of control we have over the SIP process is sufficient to produce "appropriate materials" to satisfy a target application. Below, we will use a trial and error method to produce materials for protein microarrays.

The poly(OEGMA) was an excellent nonfouling material that decreased the nonspecific protein adsorption.[24] Here we tried to use poly(OEGMA) modified iES nanofibers as the substrate for protein microarrays, which will benefit both from the large surface of nanofibers and nonfouling property of poly(OEGMA). By using the trial and error method, we identified the optimal parameters for fabricating poly(OEGMA) modified iES nanofibers for the protein microarrays as follows. The ES parameters were: PMMA concentration 6%, PMMA/A/B/C = 5 : 10 : 2 : 0.001, voltage 26 kV, and working distance of 13 cm, ES time 5 min; SIP parameters: OEGMA/$CuCl_2$/Bipy/AscA = 50 : 1 : 2 : 1, and reaction time 1 h. We used a fluorescence immunoassay to test the performance of the poly(OEGMA) modified iES nanofibers as the substrate for the protein microarrays. Before a standard sandwich immunoassay, antigen (Goat IgG) arrays were dispensed on the modified iES film by a noncontact automatic arrayer, and fixed for about 12 h. At the beginning of the immunoassay, the microarray should be totally wetted with TBST. Next, the substrate with antigen arrays was incubated, first with capture antibody (R *anti*-G IgG), and then with detection antibody (TRITC conjugated anti-Rabbit IgG) for 30 min for each incubation. The unbound antibodies were removed by 5 mL TBST flushing. After drying the microarray in a N_2 flow, we detected the fluorescent signals of the microarrays with a microarray scanner. A representative image is shown in Fig. 6a. From the results, the iES film modified with poly (OEGMA) showed the following advantages: (1) excellent signal uniformity. In the clinical diagnosis, coefficient of variation (CV = (STD/Average) × 100%) was used to judge the signal uniformity. On our iES film, CV was around 10%, which was better than many other commercial protein assay products.[25] (2) High sensitivity. The limit of detection (LOD) was about 3.78 ng mL^{-1} for IgG (Fig. 6b). (3) Good *anti*-nonspecific protein absorption ability. For most of the commonly used substrates, such as cellulose membranes, blocking process and long washing times are necessary for decreasing nonspecific adsorption. When using poly(OEGMA) modified iES films as the substrate, such procedures are no longer needed. Moreover, the background of the array hardly increased as the concentration of R *anti*-G IgG increased (Fig. 6b). (4) Time saving. The whole immunoassay takes less than 2 h (not including antigen immobilization), while the general sandwich ELISA protocol needs 3∼4 h or more.

Conclusions

In conclusion, we fabricated nanofibrous membranes with tunable surface chemistry by a general strategy for the surface modification of ES nanofibers that combined iES and SIP. This demonstration will liberate the ES community from the burdensome work of ES different materials to cater for different applications. The iPDMS/PMMA nanofibers were used here as a model scaffold. The iPDMS/PMMA nanofibers can be stored for several months yet retain its SIP reactivity and can be used directly without any pretreatment. The versatile SIP monomers enabled us to achieve application orientated surface chemistry of nanofibers, rendering the universality of this strategy. For example, we demonstrated that using poly(OEGMA) modified iES nanofibers was a superior substrate for protein microarrays, which decreased the non-specific adsorption to a very low level, improved the sensitivity and facilitated the process of immunoassays. Moreover, this method is also an effective way to fabricate nanofibers with core/shell structure. We believe that, by using this method to functionalize iES nanofibers, electrospun mats can find more applications, especially in biomedical engineering.

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