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Polypropylene non-woven meshes with conformal glycosylated layer for lectin affinity adsorption: The effect of side chain length

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ABSTRACT

The unique characteristics of polypropylene non-woven meshes (PPNWMs), like random network of overlapped fibers, multiple connected pores and overall high porosity, make them high potentials for use as separation or adsorption media. Meanwhile, carbohydrates can specifically recognize certain lectin through multivalent interactions. Therefore glycosylated PPNWMs, combing the merits of both, can be regarded as superior affinity membranes for lectin adsorption and purification. Here, we describe a versatile strategy for the glycosylation of PPNWMs. Two hydrophilic polymers with different side chain length, poly(2-hydroxyethyl methacrylate) (PHEMA) and poly(oligo(ethylene glycol) methacrylate) (POEGMA), were first conformally tethered on the polypropylene fiber surface by a modified plasma pretreatment and benzophenone (BP) entrapment UV irradiation process. Then glucose ligands were bound through the reaction between the hydroxyl group and acetyl glucose. Chemical changes of the PPNWMs surface were monitored by FT-IR/ATR. SEM pictures show that conformal glucose ligands can be achieved through the modified process. After deprotection, the glycosylated PPNWMs became superhydrophilic and had high specific recognition capability toward Concanavalin A (Con A). Static Con A adsorption experiments were further performed and the results indicate that fast adsorption kinetics and high binding capacity can be accomplished at the same time. We also found that increasing the side chain length of polymer brushes had positive effect on protein binding capacity due to improved chain mobility. Model studies suggest a multilayer adsorption behavior of Con A.

1. Introduction

Polypropylene non-woven meshes (PPNWMs) are one kind of the most popular membrane materials due to their excellent mechanical properties, high thermal and chemical stabilities, and comparatively low-cost. In addition, the random network of overlapped fibers offers many unique characteristics, including relatively large specific surface area, engineered interconnected pores and overall high porosity. Computational fluid dynamics (CFD) simulation results reveal that such disordered fibrous structure endows non-woven meshes with high permeability, low pressure drop and reduced mass transfer/diffusion resistance [1]. Therefore, PPNWMs exhibit promising prospects for comprehensive applications in air/liquid filtration or adsorption media, biomedical textile and protective cloth [2–6].

Among these promising applications of PPNWMs, membrane chromatography for protein bioseparation has captured growing attention in recent years [3,7–10]. Industrial protein purification process is conventionally carried out using packed bed or column chromatography, which has several major limitations such as slow intraparticle diffusion, large pressure drop, high cost and hence low protein binding capacity/productivity and difficulty in scale-up. The unique structural characteristics of PPNWMs make them the most promising alternative materials to replace traditional packed-bed resins [11,12]. For example, PPNWMs grafted with conformal anion exchange ligands and hydrophilic spacers were prepared through a UV pretreatment-UV grafting process, and it was found that such activated meshes had high bovine serum albumin binding capacity and permeability coefficient [9]. Even when stacking 150 layers in a column, the pressure drop was still acceptable and it helped to create sharp chromatographic elution profiles [13]. It was also reported that the presence of a molecular spacer between the ligand and the membrane matrix could be beneficial for protein adsorption [14]. Further attempts to increase the binding capacity led to the creation of PPNWMs supported/macroporous gel based 3D binding domain composite membrane [10]. However,

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the separation mechanism was mainly based on non-specific interactions like electrostatic attraction/repulsion, hydrophobic interaction and Vander Waals forces. It is a logical expectation that the introduction of affinity ligands with specific interaction with target proteins can result in high separation efficiency and purity.

Carbohydrates are widely regarded as energy sources and building elements. With the development of glycobiology, their structurally and functionally diverse roles are gradually discovered [15]. Carbohydrate–protein recognition mediates various biological processes and is the first step in cell–cell interactions *via* multivalent interactions, or glycocluster effect [16]. Lectin, as a kind of carbohydrate-binding proteins or glycoproteins, can specifically recognize certain carbohydrate moieties [17]. Meanwhile, carbohydrates are highly hydrophilic molecules that non-specific protein adsorptions can be greatly reduced or eliminated. Therefore, glycosylated PPNWMs are reasonably expected as superior affinity membranes for lectin adsorption and purification. In the literature, a few studies have reported the fabrication of glycosylated poly(ethylene terephthalate) fibers [18–20]. However, it is rarely addressed on the bioseparation performance of glycosylated non-woven meshes.

In this work, our goal is to develop a facile method for the construction of glycosylated PPNWMs and further evaluate their application prospects as affinity membranes for lectin purification. First, a modified plasma pretreatment and benzophenone entrapment UV irradiation process was employed to graft conformal and uniform poly(oligo(ethylene glycol) methacrylate) and poly(2-hydroxyethyl methacrylate) (POEGMA and PHEMA) brushes on the fiber surfaces of PPNWMs. Then surface glycosylation was accomplished by using different acetyl glycosyl donors in the presence of boron trifluoride diethyl etherate and subsequent deprotection. The surface wettability, qualitative fluorescein-labeled lectin and quantitative static Con A adsorption were characterized. Furthermore, the effect of side chain length on adsorption capacity was also evaluated.

2. Experimental

2.1. Materials

Commercial PPNWMs (Jiangyin Golden Phoenix Special Textile Co., Ltd, China) used in this work were produced with a melt-blown process. The fiber diameter was in the range of 5–10 μm. The density and porosity were about 35 g/m^2 and 80%, respectively. The samples were cut into rotundity with a diameter of 2.5 cm (S = 4.91 cm^2), washed with acetone and dried in a vacuum oven at 40 °C. 2-Hydroxyethyl methacrylate (HEMA, 97%) and oligo(ethylene glycol) methacrylate (OEGMA, M_n = 360, 99%) were supplied by Sigma–Aldrich and passed through neutral Al_2O_3 flash column chromatography to remove inhibitors. Benzophenone (BP) was recrystallized from cold ethanol. Boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) was purified by vacuum distillation. Dichloromethane was distilled from phosphorus pentoxide immediately before use. Trichloroacetonitrile, 2,3,4,6-tetraacetyl-β-D-glucose and β-D-glucose pentaacetate were bought from Beijing Chemsynlab Pharmaceutical Science & Technology Co. Ltd. and used as received. Concanavalin A (Con A), fluorescein-labeled Con A (FL-Con A) and peanut agglutinin (FL-PNA) (Vector, USA) were purchased and used directly. All the other chemicals like sodium methoxide, heptane, petroleum ether (60–70 °C), potassium carbonate, and 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) were of analytical grade and used without further purification. Water used in all experiments was deionized and ultrafiltrated to 18 MΩ using an ELGA LabWater system (ELGA Classic UF, France).

2.2. Graft polymerization of OEGMA and HEMA onto PPNWMs

The experimental procedure is schematically illustrated in Fig. 1. Dielectric barrier discharge plasma was used to pretreat the nascent PPNWM at atmospheric pressure by a plasma apparatus (Nanjing Suman Electronics Co., Ltd., China). Two quartz glass plates with a gap of 2 mm served as the dielectric layer and argon (99%)/air (1%) was used as the discharge gas. Firstly, the sample was irradiated at 35 V and 10 kHz for a given time and exposed in the air for 10 min. After that, the pretreated PPNWMs were immersed in 20 mM BP heptane solution for 45 min to immobilize the photoinitiator in the surface layer of the polypropylene fiber and then dried in the air. Thereafter, the PPNWMs were presoaked with acetone and immediately dipped into OEGMA or HEMA solutions in petri dishes and fixed between two sheets of filter paper. Finally, UV grafting polymerization on the surfaces of PPNWMs was carried out under a homemade high pressure mercury lamp (232–400 nm, intensity 3 mW/cm^2) for a predetermined time. The grafted PPNWMs were washed thoroughly with ethanol overnight to remove unreacted monomer and homopolymer, and then dried in a vacuum oven at 40 °C. They were weighed with an analytical balance to a precision of 0.01 mg (XP105DR, Mettler Toledo, Switzerland). The grafting density (GD, μg/cm^2) was calculated by the following equation:

$$GD = \frac{W_1 - W_0}{S} \quad (1)$$

where W_0 and W_1 are the mass of the nascent and the POEGMA/PHEMA grafted PPNWMs, respectively. S represents the surface area of each sample.

2.3. Coupling of glucose ligands to hydroxyl groups of the POEGMA/PHEMA grafted PPNWMs

Surface glycosylation reaction was conducted as reported in our previous work [21]. Three pieces of POEGMA/PHEMA grafted PPNWMs were fully immersed in 6 mL freshly dried dichloromethane solution containing 20 equiv glucose pentaacetate and 100 equiv $BF_3{\cdot}Et_2O$. The reaction was sealed in a Schlenk tube and carried out at 0 °C for 2 h followed by 20 h at room temperature. After that, the samples were washed extensively with ethanol and dried in a vacuum oven at 40 °C.

Glycosyl trichloroacetimidate exhibiting excellent glycosyl donor properties was also tested, and the general synthesis procedure was as follows [22]. 2,3,4,6-Tetraacetyl-β-D-glucose (2 g) was dissolved in freshly dried dichloromethane (20 mL), and treated with trichloroacetonitrile (4 mL) and finely powdered K_2CO_3 (2 g). The solution was stirred at room temperature and monitored by thin layer chromatography (TLC) till the complete consumption of the raw material. The reaction mixture was filtered and concentrated. Crude product was further purified by column chromatography [ethy1 acetate–petroleum ether (1:2, v/v)] to afford 2,3,4,6-tetraacetyl-α-D-glucopyranosy trichloroacetimidate as a yellow syrup in yield of 80.3% ($[\alpha]^{20}_D$ = +92.5 ($CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm):8.69 (s, 1H, C(NH)CCl_3), 6.56 (d, 1H, J = 3.6 Hz, H-1), 5.56 (t, 1H, H-3), 5.18(t, 1H, H-4), 5.13 (dd, 1H, H-2), 4.25–4.12 (m, 3H, H-5, H-6), 2.07(s, 3H, oAc), 2.04 (s, 3H, oAc), 2.03(s, 3H, oAc), 2.01(s, 3H, oAc)). The glycosylation procedure was the same as described before. The binding density (BD, μg/cm^2) of glucose ligands and the reaction ratio (R, %) of hydroxyl groups were calculated according to the following equations:

$$BD = \frac{W_2 - W_1}{S} \quad (2)$$

$$R = \frac{M_n(W_2 - W_1)}{330(W_1 - W_0)} \times 100\% \quad (3)$$