Journal of Membrane Science 428 (2013) 562–575

Contents lists available at SciVerse ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Affinity membrane development from PBT nonwoven by photo-induced graft polymerization, hydrophilization and ligand attachment

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ARTICLE INFO

Article history:
Received 25 July 2012
Accepted 16 September 2012
Available online 22 October 2012

Keywords:
Affinity membrane
Functional nonwoven fabrics
Surface modification
Protein purification
Nonspecific protein adsorption

ABSTRACT

Nonwoven fabrics are of great interest as potential materials for bioseparations due to their interconnected porous structure, relatively high surface area and low cost. In this paper we focus on the development of a potentially disposable affinity membrane for pathogen removal from biological systems such as human plasma. Poly glycidyl methacrylate (polyGMA) was grafted on the fiber surface of a polybutylene terephthalate (PBT) nonwoven using photo-induced graft polymerization. SEM and FTIR were used to characterize the pore structure and surface chemistry of the resulting material. To minimize nonspecific protein binding and hydrophilize the material, diethylene glycol (DEG) and diol groups were attached covalently to the grafted layer of polyGMA. The amount of nonspecific binding was quantified by the adsorption of bovine serum albumin (BSA) and an *E. coli* extract. The results showed that the grafted matrix containing DEG or diol groups bound significantly less total protein, compared with unmodified material. The DEG modified membrane was further developed by attachment of a specific proprietary ligand that binds to the prion protein, the agent responsible for transmissible spongiform encephalopathies. The affinity membrane showed good selectivity for the capture of prion protein from hamster brain homogenate.

1. Introduction

Nonwoven fabrics possess many unique characteristics, including engineered interconnected porous structure, relatively large surface-to-volume ratio, controllable pore size, porosity, fiber diameter, fiber shape and low production cost. Porous nonwoven fabrics have drawn increasing interest as new materials for applications in the fields of bioengineering and bioprocessing, such as wound dressings, 3-D scaffolds for bone and cartilage regeneration, drug delivery carriers and liquid filtration and separation devices [1–8].

Among the numerous potential applications of nonwoven membranes, one of them is downstream processing for protein separation and purification, which is traditionally carried out in packed-bed chromatography columns filled with porous beads owing to their high separation efficiency. Conventional bead-based column chromatography exhibits drawbacks such as slow intraparticle diffusion, large pressure drop through the column, low product throughput, high cost and difficulty in scale-up. The diffusional limitations can cause reduced dynamic binding capacities for macromolecules, especially for large targets with molecular weights greater than 250 kDa [9]. Chromatography columns with long flow paths result in relatively low throughputs because the pressure drop becomes very high at high flow rates [10,11]. This leads to the use of large columns that can be very wide (several feet) and relatively shallow but are also difficult to pack uniformly.

As a result, the biopharmaceutical industry is seeking alternative materials to replace the traditional packed-bed columns [12–16]. Among these methods, membrane chromatography can overcome some of these limitations because ligands are attached to the pore surfaces in the membrane and convective flow can bring the solutes to the ligand-modified surfaces directly, thus eliminating diffusional resistances in the product adsorption step. The pressure drop across membrane devices tends to be lower than in packed beds due to the much short flow paths and large surface areas associated with most common membrane device configurations. This can result in higher superficial velocities and higher productivities for membrane chromatography when compared to packed-bed chromatography. The relatively easy scale-up of membrane chromatography also provides additional advantages [17–21].

In order to reduce the costs of production of protein biotherapeutics, the industry is moving to disposable bioreactors

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http://dx.doi.org/10.1016/j.memsci.2012.09.047

utilizing pre-sterilized packages for cell culture of products such as monoclonal antibodies (mAbs). These plastic containers can be disposed after single use, thus eliminating cleaning-in-place, sterilization-in-place and validation processes required with the use of stainless steel bioreactors [22]. Unfortunately, chromatographic cast membrane devices are too expensive to be tossed away after single use, and little progress has been made in the development of membrane chromatography systems that can be completely disposable. Such devices would provide a real breakthrough in the industry and result in significant cost reductions, especially when applied to product capture chromatography as well as pathogen and contaminant reduction. The cost of nonwoven fabrics, especially commercially available nonwoven materials such as polypropylene, polyesters, and nylon, is much lower than that of cast membranes. As a result, nonwoven fabrics offer a good opportunity to develop disposable membrane chromatography to be used for both product capture or pathogen removal.

Despite nonwoven membranes having great potential for the use as disposable membrane chromatography, as far as we are aware, there are no commercially available nonwoven functional membranes for product or impurity capture. In the academic arena, the idea of making functional membranes from nonwovens has drawn considerable attention during the last two decades. For example, Kim and co-workers developed cation-exchange membranes from nonwoven fabrics composed of polyethylene (PE) and polypropylene (PP) microfibers (diameter of $\sim$30 μm) with a two-step procedure: radiation-induced (electron beam) graft polymerization and subsequent ligand attachment [23]. Ma et al. developed affinity membranes with dye ligands, protein A and protein G on electrospun polysulphone or cellulose membranes via surface grafts initialized by plasma or oxidization treatments [24–26]. Matsumoto and co-workers developed cation- and anion-exchange fiber fabrics by sulfonation of polystyrene fibers and the quaternization of poly (4-vinylpyridine) fibers, respectively [27]. Zhang et al. developed anion-exchange adsorptive membranes by introducing diethylaminoethyl (DEAE) onto regenerated cellulose nanofibers by chemical treatments [28]. Menkhaus and co-workers achieved an adsorption medium with ultra-high protein binding capacity from electrospun regenerated cellulose nanofibers by atom transfer radical polymerization (ATRP) [29].

So far, little effort has been focused on the use of UV grafting methods to convert nonwovens into functional membranes for bioseparations. Comparing with other surface modification methods such as plasma treatment, high-energy electron beam and γ-ray irradiation, UV techniques do not require very complex or expensive equipments, and are relatively easy to operate and maintain [30–33]. In addition, UV irradiation can be very effective in introducing functional groups onto polymeric material surfaces without damaging the bulk material [30,31]. Benzophenone (BP) has been widely used as a photoinitiator in photo-induced surface grafting to initialize free radical polymerization by hydrogen abstraction under the influence of UV light [34]. In a previous publication, our group has demonstrated the formation of highly conformal and uniform polyGMA grafts on PP nonwoven fabrics using a UV pretreatment step followed by a UV grafting step in the presence of BP as an initiator [35]. This paper describes the formation of polyGMA grafted layers on a PBT nonwoven using a single UV grafting step to achieve a polyGMA-grafted membrane (PBT-GMA). A commercial PBT nonwoven membrane has been selected for its low manufacturing cost, high tensile strength, heat stability, and also because it is relatively easy to modify compared with polyolefins. In addition, PBT has been approved for use in membrane devices that come in contact with blood and blood products [36], and our primary interest is in the development of a disposable membrane for prion protein removal from blood. GMA was used as the grafting monomer because it is a common and versatile monomer with a reactive epoxy end group which can be used to introduce different functional groups to achieve ion exchange or affinity media with additional reaction steps [37]. For example, hydroxyl (OH), diethylamino (D), strong basic quaternary ammonium (Q), sulfonic acid (S) and carboxylic acid (C) groups can be obtained by means of various reagents [37,38]. For affinity media preparation, different activation methods can be used to introduce different ligands from epoxy groups directly or hydroxyl, amino or carboxyl derivatives [17,39].

Most commercial nonwoven materials, such as polyolefins and polyesters, are hydrophobic. However, hydrophobicity is not a desirable property for selective protein separations, because many proteins tend to adsorb onto the hydrophobic fiber surface non-selectively when these nonwoven fabrics are in contact with a protein solution. In bioseparation applications, it is critical to get a conformal grafted layer of hydrophilic, neutral material with functional groups around the original hydrophobic fibers to reduce nonspecific protein binding and to maintain the porous structure after surface modification. Oligo/poly (ethylene glycol) (OEG/PEG) is widely used to resist protein adsorption on different surfaces, including polymer, silver, gold, graphite, and silica [40]. It is believed that both steric effects and chain hydration play an important role in the ability of a OEG/PEG layer to reduce nonspecific binding of proteins [41]. Moreover, the hydroxyl end group of OEG/PEG can also be used to react with other chemicals to introduce affinity or ion-exchange ligands [37]. In the work described in this paper, diethylene glycol (DEG) was used to impart protein resistance and hydrophilicity to the surface after polyGMA grafting. Its effect was compared to that of the membrane with diol groups resulting from the hydrolysis of the grafted polyGMA. Measurements on the nonspecific adsorption of proteins on these hydrophilized nonwoven membranes with different degree of GMA grafting were carried out after DEG and diol modifications. For additional comparison, this work also included measurements of nonspecific protein binding to the PBT nonwoven membranes treated by UV/ozone and plasma. In industry, UV and plasma treatments are commonly used to increase the hydrophilicity of hydrophobic surfaces such as PBT. Both the model protein bovine serum albumin (BSA) and a much more complex *E. coli* extract were used to demonstrate the nonspecific protein binding reduction with our membranes after two-step surface treatments (GMA grafting and DEG or diol attachments). To determine the structural changes of the nonwoven membranes as a result of the surface modification steps, the mean flow pore size, surface morphology, and permeability of the nonwoven samples were determined before and after surface treatments.

In addition, a proprietary affinity ligand was attached to the DEG modified PBT-GMA to create an affinity membrane. This ligand has been shown to bind infectious prions from red blood cell concentrates as well as capture endogenous prion infectivity from whole blood using an animal model [42,43]. The ability of this affinity membrane to capture prion protein selectively from hamster brain homogenate was determined.

The results in this paper demonstrate that UV grafting can generate very conformal grafts on the PBT fiber surfaces with insignificant changes on the porous structure. Nonspecific protein binding of BSA and the *E. coli* extract were significantly reduced after hydrophilization with DEG or diol. The affinity membrane for prion capture was able to selectively remove prions from a complex mixture with a high binding capacity. This work demonstrates that nonwoven fabrics can provide a promising alternative for the development of disposable membranes for pathogen capture and removal.

2. Experimental

2.1. Materials and reagents

PBT nonwoven was provided by Macopharma (Tourcoing, France), and has a base weight of 52 g/m^2. Glycidyl methacrylate and benzophenone were purchased from Sigma-Aldrich (St. Louis, MO). Inhibitors in GMA were removed through a pre-packed inhibitor remover column prior to use (Sigma-Aldrich, St. Louis, MO). Methanol, 1-butanol (A.C.S. grade), tetrahydrofuran (THF), and diethylene glycol (DEG) were all purchased from Fisher Scientific (Fairlawn, NJ). Sulfuric acid was from Acros (Morris Plains, NJ).

Albumin from bovine serum (BSA), lysozyme, β-casein and mouse immunoglobulin G (MIgG) were purchased from Sigma-Aldrich (St. Louis, MO). Human immunoglobulin G (hIgG) was purchased from Equiteck-Bio Inc. (Kerrville, TX). All these proteins were used as received. *E. coli* extract for green fluorescent protein (GFP) production was obtained from the Biomanufacturing Training and Education Center (BTEC) at NC State University after homogenization, centrifugation and filtration.

Hamster brain homogenate (10% w/v) and affinity resin for prion protein capture were provided by PRDT (Pathogen Removal and Diagnostic Technologies, Inc., a subsidiary of ProMetic Biosciences, LTD, Cambridge, UK). Hamster brain homogenate was stored at -80 °C before use. PIKSI columns were provided by ProMetic BioSciences, Ltd. (Cambridge, UK). NuPAGE gel, PVDF (polyvinylidene fluoride) membrane, pre-stained protein standard, sample buffer, reducing agent, MES SDS running buffer, NuPAGE antioxidant and transfer buffer were purchased from Invitrogen (Carlsbad, CA). Mouse anti-prion protein monoclonal antibody 3F4 was purchased from Signet Laboratories (Dedham, MA). Goat anti-mouse alkaline phosphatase-labeled antibody (secondary antibody) was purchased from KPL, Inc. (Gaithersburg, MD). The Western blot immunodetection kit Western Breeze purchased from Invitrogen was used for chemiluminenscent labeling. The X-ray film used to visualize the Western blots was purchased from Amersham Biosciences (Little Chalfont, UK).

2.2. UV-induced graft polymerization of GMA on PBT nonwoven

Inhibitor in GMA was removed by flowing 500 ml GMA under gravity through a pre-packed inhibitor remover column prior to use. GMA solutions were prepared using 1-butanol as solvent at three GMA concentrations of 0.75 M, 1.5 M and 2.0 M, followed by the addition of the photoinitiator BP at different BP:GMA ratios in the range of 0:100 to 32:100. Nonwoven membrane samples were washed with methanol and dried in a vacuum oven at 30 °C before use. Pre-weighed nonwoven pieces (75×50 mm^2) were wetted by spraying the GMA solution evenly until the material was completely soaked, followed by sandwiching the wet membrane between two glass slides (75×50 mm^2). One side of the sample was exposed to a UV lamp (model EN-180L, Spectronics Corporation, Westbury, NY) with wavelength centered at 365 nm and an intensity of 5 mW/cm^2. The distance between the nonwoven samples and the lamp was 3 mm.

After a determined reaction time in the range of 5 to 30 min, the residual, unreacted solution and GMA homopolymer were removed by soaking and washing the membrane in THF for 30 minutes in an ultrasonic bath (Bransonic 3510R-MT, Branson Ultrasonics Corporation, Danbury, CT) at room temperature followed by two methanol washes of 10 min each. After the THF and methanol washes, the sample was also extracted extensively with acetone in a Soxhlet apparatus for 20 h. Before and after soxhlet extraction, the membrane was dried in a vacuum oven at 30 °C for 5 h. The nonwoven membrane was weighed to determine the degree of GMA grafting (percent weight gain), which was calculated using the expression,

$$\text{Degree of GMA grafting } (\%) = \frac{W_1 - W_0}{W_0} \times 100\% \quad (1)$$

Here W_0 is the original PBT nonwoven weight and W_1 is the weight after polyGMA grafting.

2.3. Hydrophilization: DEG attachment and hydrolysis

The polyGMA grafted nonwoven materials (PBT-GMA) were placed in diethylene glycol (DEG) with catalytic amounts of sulfuric acid (around 0.1 M) at 50 °C for 8 h on a shaker at 100 rpm. The resulting DEG-treated nonwovens (PBT-GMA-DEG) were washed with deionized water five times in a sonication water bath at room temperature for 5 min each wash. Samples were then dried in a vacuum oven at 30 °C for 5 h.

Samples of PBT-GMA were also put into 0.1 M sulfuric acid solution at 50 °C overnight to hydrolyze the sample by reacting the epoxy groups with water to convert the epoxy groups to diols (PBT-GMA-diol), followed by the same wash and drying procedure as after attachment of DEG as described above.

2.4. Material characterization

The surface chemical compositions of unmodified and modified PBT nonwoven membranes were characterized by attenuated total reflectance Fourier transform infrared (ATR-FTIR) using a Nicolet spectrometer with a smart OMNI-Sampler accessory (Thermo-Nicolet Nexus 470-FTIR, Thermo Fisher Scientific, Waltham, MA). Each spectrum was collected by absorbance with 64 scans at a resolution of 4 cm^{-1}. The beam radius was 5 mm with inverse wavelength 4000–675 cm^{-1}. The depth of penetration was 0.67 μm at 2000 cm^{-1}.

For physical property characterization of nonwoven membranes, scanning electron microscopy (SEM) images were obtained using a Hitachi S-3200N variable pressure microscope (Hitachi High Technologies America, Inc., Schaumburg, IL). The accelerating voltage was 5 kV with a working distance around 22 mm. Nonwoven samples were fixed onto a sample holder by carbon tape and sputtered with Pd/Au alloy in argon gas before they were put into the microscopy chamber.

The contact angles of blank PBT and hydrophilized samples of PBT-GMA-DEG and PBT-GMA-diol were measured with a standard goniometer (Model No. 200-F1, Rame-Hart Instrument Co., Netcong, NJ). The hydrostatic penetration pressures of the blank PBT, PBT-GMA-DEG and PBT-GMA-diol membranes were measured with a hydrostatic head tester Textest FX3000 (Textest AG, Switzerland) by pushing deionized water through one layer of nonwoven membrane. The mean flow pore size of the nonwoven membrane was measured with a capillary flow porometer (Model No. CFP-1100-AX, Porous Materials Inc, Ithaca, NY).

The average fiber diameter was calculated by analyzing 200 fibers from the SEM images. The nonwoven membrane layer thickness was also measured from SEM membrane cross-section images. The specific surface area of the membrane was measured using an Autosorb^{TM}-1C chemisorption-physisorption analyzer (Quantachrome Instruments, Boynton Beach, FL). The single-layer porosity was calculated by the expression,

$$\text{Porosity} = \left(1 - \frac{\rho}{\rho_p}\right) \times 100\% \quad (2)$$

Here ρ_p is the bulk PBT density, which is 1.31 g/cm^3 [44] and ρ is the apparent density (g/cm^3) of the PBT nonwoven membrane. The apparent density was obtained by dividing the dry mass by its volume, which was obtained by multiplying the

membrane area and the measured membrane thickness (300 μm). The base weight of the membrane is 52 g/m^2.

For flow permeability measurements, the pressure drop of a nonwoven membrane packed column was determined at 7 different flow superficial velocities from 5.8 cm/min to 0.24 cm/min with DI water. The nonwoven membranes were cut into discs with diameter of 2.2 cm, weighed, wetted with 20% (v/v) methanol and packed in a column. 0.65 g material was packed with a column height of 0.5 cm for the PBT nonwoven membranes before and after surface hydrophilization with a packing density of 0.34 g/ml. The column, a Vantage chromatography column (EMD Millipore, Billerica, MA), was connected to a peristaltic pump (DynamaxR Rainin, Oakland, CA), which controls the flow superficial velocities. The superficial velocity was calculated by dividing the collected water volume by the product of collecting time and the cross-section area of the column. The pressure at each end of the column was measured by a digital pressure gauge with an accuracy of ± 0.02 bar (Cole Parmer Instrument Co., Vernon Hills, IL). The pressure drop at different flow superficial velocities without membrane packed in the column was also measured. The pressure drop caused by the membrane stack was calculated by subtracting the pressure drop without membrane packed in the column from the pressure drop with membranes at the same flow rate.

2.5. Nonspecific protein binding

Nonwoven samples of PBT-GMA-DEG and PBT-GMA-diol with different degrees of GMA grafting were first used to determine the impact of the degree of GMA grafting on the nonspecific binding of BSA as a model protein. The nonwoven membranes have the same DEG or diol treatment but different degrees of GMA grafting in the range of 0 to 20%. Around 0.2 g of nonwoven membrane sample was put into approximately 3 ml of BSA solution with an original concentration of 0.5 mg/ml for 12 h at room temperature under shaking at 100 rpm. The protein solution was prepared with pH 7.4 PBS buffer (0.01 M phosphate, 0.138 M NaCl, 0.0027 M KCl). The membrane was also put into binding buffer alone as a control. Protein concentrations in solution before and after binding were measured using the bicinchoninic acid method (BCA™ protein assay kit, Pierce, Rockford, IL) or spectrophotometry at 280 nm (Agilent 8453, Agilent Technologies, Santa Clara, CA).

The amount of protein bound to the nonwoven membrane samples was calculated using the expression,

$$\text{Amount of protein adsorbed} = \frac{(C_0 - C_1) \times V}{W} \quad (3)$$

Here C_0 and C_1 are protein concentration before and after binding, respectively (mg/ml), V is protein solution volume (ml), W is nonwoven weight (g).

Once the impact of the degrees of GMA grafting on BSA binding was studied, membranes with 12% degree of GMA grafting were selected as the grafting level for the following studies. Protein binding of blank PBT, PBT-GMA, PBT-GMA-DEG and PBT-GMA-diol membranes were performed to determine if the resistance to protein adsorption derives from the polyGMA grafted layer or the hydrophilized grafted layer. BSA solution and binding conditions are the same as mentioned above.

Static BSA binding isotherms of surface hydrophilized nonwoven membranes (PBT-GMA-DEG and PBT-GMA-diol), blank PBT, UV/ozone-treated and plasma-treated PBT were studied with BSA solutions at concentrations ranging from 0.1 mg/ml to 3 mg/ml in PBS buffer. The UV/ozone-treated PBT membrane was prepared by exposing the membrane to UV for 5 min per side (UV/ozone cleanser No.342, Jelight Company Inc, Irvine, CA). The argon plasma-treated PBT nonwoven was provided by MacoPharma (Tourcoing, France).

The ability of the hydrophilized materials PBT-GMA-DEG and PBT-GMA-diol to bind lysozyme, hIgG and β-casein were also investigated, using protein solutions of 2 mg/ml in PBS buffer at pH 7.4. Around 0.2 g of the blank PBT, PBT-GMA-DEG or PBT-GMA-diol membranes were put into 3 ml of protein solution for 12 h at room temperature under shaking at 100 rpm.

Samples of nonwoven membrane were cut into discs with diameter of 0.9 cm and 20 layers of the membrane were packed into PIKSI kit columns at height of 0.4 cm, with two frits on the top and bottom to hold the membrane bed at a fixed position. PIKSI kit is a plastic multi-column kit containing 10 columns with column diameter of 0.8–0.9 cm and column height of 2 cm. Nonwoven membranes studied here include original blank PBT, UV/ozone-treated PBT, plasma-treated PBT, PBT-GMA-DEG and PBT-GMA-diol in duplicate columns. The membrane packed columns were challenged with an *E. coli* extract that was homogenized with cell disruption, followed by centrifugation for removal of cell debris, and filtered with a 0.22 μm filter. For each membrane column, 10 ml of *E. coli* extract in 50 mM Tris buffer at pH 8.0 was passed through the column using a peristaltic pump set at 1 ml/min. Thereafter, all the membranes were recovered from the column and washed three times with 50 mM Tris buffer at pH 8.0. Then, the bound protein was extracted by 0.5 ml 2X invitrogen sample buffer (prepared by 4X Invitrogen NuPage sample buffer with Invitrogen NuPage sample reducing agent) by heating at 90 °C for 10 min. This step removed the bound proteins from the membranes surface to facilitate further analysis. The SDS-PAGE was performed with Invitrogen NuPage 4–12% Bis-Tris 10-well gel with 20 μl of extracted sample for each channel. The gels were run at constant 200 V for approximately 35 min until dye front was within 0.5 cm of gel bottom. The gel was stained with the Coomassie Brilliant Blue.

2.6. Prion protein capture with affinity membrane

A proprietary ligand with affinity to prion proteins [43] was attached to the hydroxyl groups from PBT-GMA-DEG to achieve an affinity membrane for prion capture. The affinity membrane was cut into discs with a diameter of 9 mm, and 18 discs were stacked and packed into a PIKSI column. An affinity resin containing the same ligand as the membrane (ProMetic Biosciences, Ltd., Cambridge, UK), and two samples of nonwoven membrane containing hydroxyl groups (PBT-GMA-DEG and PBT-GMA-diol) were also packed into PIKSI columns. All packed columns had the same final packed column height of 0.4 cm. The affinity membrane and the affinity resin were tested in duplicate, while only one column of PBT-GMA-DEG and PBT-GMA-diol were tested. The membranes and resins were tested against a hamster brain homogenate (HaBH) solution. The stock solution of 10% brain homogenate was thawed, and 5% Sarkosyl (Sigma-Aldrich, St. Louis, MO) reagent was added to thawed brain homogenate, to a final concentration of 0.5%, followed by incubation for 30 min under gentle mixing. The brain homogenate was then centrifuged at 14,000 rpm for 5 min at 4 °C, and the supernatant was carefully collected for use. A 1% brain homogenate was then prepared by diluting the Sarkosyl-treated solution with working buffer (20 mM citrate buffer and 140 mM NaCl at pH 7.0). Six milliliters of 1% HaBH were applied to each of the columns at a flow rate of 0.5 ml/min using a peristaltic pump. After challenge, the columns were unpacked, and the membranes and resin were transferred to 15-ml conical tubes for washing with working buffer at least twice. All the resin was allowed to settle, and the washing solutions were removed, followed by the addition of 0.5 ml 2X SDS-PAGE sample buffer (Invitrogen, Carlsbad, CA).

The membranes were washed with working buffer and treated with the sample buffer in the same way. Then the samples were heated at 90 °C for 10 min, followed by a quick centrifugation. This step removed the bound protein from the membranes or resins to facilitate further analysis. The supernatants were analyzed by SDS-PAGE and Western blot.

For the electrophoresis, NuPAGE 1X reduced electrophoresis running buffer was prepared by mixing 20X NuPAGE MES running buffer with DI water. Immediately prior the run, 500 µl of Invitrogen NuPAGE antioxidant was put into inner buffer chamber. Invitrogen electroblot transfer buffer was prepared by mixing 100 ml of Invitrogen 20X NuPAGE transfer buffer with 1500 ml DI water and 400 ml methanol to a total volume of 2 l. Two milliliters of Invitrogen NuPAGE antioxidant was added and the solution was chilled prior to use. Two Invitrogen NuPAGE 12% Bis-Tris gels were used for SDS-PAGE, with one gel stained for total proteins, and the other gel used for Western blot for prion protein analysis. A control solution of 1.35% HaBH in sample buffer was prepared and applied to the gels. Low-, medium- and high-concentration mouse immunoglobulin G (MIgG) was loaded onto the gels to be used as weight marker with 2 ng/lane, 10 ng/lane and 50 ng/lane. All samples, including the controls, were heated at 90 °C for 10 minutes before being applied to the SDS PAGE gels. A pre-stained protein standard was used as molecular marker without heat treatment. 10 µl of each sample, and 5 µl of molecular weight marker were loaded into the gels.

After running the gels, one was stained with Coomassie Brilliant Blue stain, while the other was transferred to an Invitrogen PVDF membrane using a BioRad electroblot chamber at a constant 100 V for 45 min. The PVDF membrane was treated with Western Breeze blocking agent for 1 h at room temperature, incubated with primary antibody Signet 3F4 overnight, under refrigeration, on a rocking platform, rinsed three times in 20 ml of Western Breeze antibody wash, incubated with secondary antibody goat-anti-mouse (GAM) for 1 hour at room temperature on a rocking platform, rinsed again three times and finally washed with 20 mM Tris-HCl plus 1 mM $MgCl_2$ at pH 9.8 for 10 min at room temperature. The membrane was transferred to a dry tray and soaked in Western Breeze pre-mixed chemiluminescence substrate for 5 min under gentle agitation. Excess solution was gently removed by wicking using paper towels. The membrane was then placed in a plastic sheet protector, and allowed to rest for 30 min, followed by exposure of an X-ray film for 5 min.

3. Results and discussion

3.1. Optimization of GMA grafting on PBT

The physical properties of the PBT nonwoven membrane used in the experiments are summarized in Table 1. The average fiber diameter was determined to be 3 µm and the membrane thickness was about 300 µm. The base weight of 52 g/m^2 provided by the membrane manufacturer was used to calculate the membrane apparent density and porosity, yielding values of 0.173 g/cm^3 and 87%, respectively. The specific surface area was determined to be around 0.72 m^2/g, after performing BET nitrogen adsorption measurements in triplicate. The mean flow pore size of the nonwoven membrane was about 8.0 ± 0.5 µm, measured with a capillary flow porometer.

Table 1
Physical properties of meltblown PBT nonwoven membrane.

Fiber diameter (µm)	3.0 ± 0.7
Mean flow pore size (µm)	8.0 ± 0.5
Membrane thickness (µm)	300 ± 30
Basis weight (g/m^2)	52
Apparent density (g/cm^3)	0.17
Porosity (%)	87
Specific surface area (m^2/g)	0.72 ± 0.10

Different concentrations of GMA monomer and photoinitiator BP were used in the grafting step and the effect of these variables on the degree of GMA grafting and grafting layer uniformity were characterized. Three different GMA concentrations were studied at 0.75 M, 1.5 M and 2.0 M with different BP to GMA ratios ranging from 0:100 to 32:100 at a fixed grafting time of 15 min. After grafting, the membrane was ultrasonically washed with THF and methanol, followed by a Soxhlet extraction with acetone. The weights of blank PBT, a dried sample of PBT-GMA after THF and methanol wash, and dried sample after Soxhlet extraction were all measured. The weights of the membranes after Soxhlet extraction were similar to the weights obtained for the materials after THF and methanol washes (data not shown), indicating that the THF and methanol washes were sufficient to remove unreacted GMA and any formed homopolymer from the material. In later experiments, the washes following grafting were reduced to THF in the ultrasound bath, followed by two methanol washes. By applying the weight values obtained for blank PBT and PBT-GMA membranes after THF and methanol washes to Eq. (1), the degree of GMA grafting for each of the different grafting conditions was determined and shown in Fig. 1. As expected, no grafting was observed when the concentration of BP was zero (BP:GMA=0:100) for all three GMA concentrations after 15 min grafting, as there was no initiator to start the process. For the three GMA concentrations tested, the degree of GMA grafting display a sharp increase when the BP to GMA ratio increases from 0:100 to 1:100, with the degrees of GMA grafting peaking at 2%, 9%, and 10.3% with GMA concentrations of 0.75 M, 1.5 M, and 2.0 M, respectively. The larger the number of BP molecules in the solution, the larger the number of radical sites on the fiber surface that can be created, resulting in higher polyGMA grafting chain densities. However, as the ratio of BP to GMA keeps increasing from 1:100 to 32:100, the degree of GMA grafting decreases rapidly. The reason for this is that a large excess of BP that cannot adsorb to the fiber surface can absorb UV light and enhance the formation of GMA homopolymer in solution, and this takes away from GMA that can be grafted to the fiber surface. In addition, more activated BP free radicals in the solution might also terminate more polymer chains growing on the fiber surface because these radicals have a very short lifetime and prefer to terminate or combine growing chains. A similar behavior was observed by Ma et al. [45], where the grafting density of surface initiator BP on porous PP membranes initially increased and then

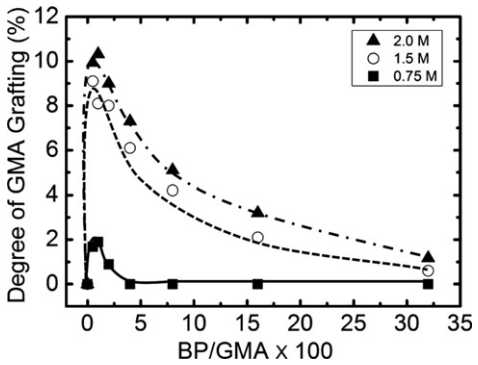

Fig. 1. Degree of GMA grafting with different GMA concentrations (0.75 M (■), 1.5 M (○), 2.0 M (▲)) and different ratios of BP to GMA (BP/GMA ranging from 0:100 to 32:100) with 15 min grafting.

decreased with increasing BP concentration when the PP membrane was immersed in benzene solution of BP with UV irradiation.

From the results in Fig. 1 it can also be observed that both GMA concentration and BP concentration have a marked effect on GMA grafting on PBT nonwoven fibers. When the GMA concentration increases from 0.75 M to 1.5 M and 2.0 M, the achieved highest degree of GMA grafting increases. Because of these results, a grafting solution with a 2.0 M GMA was used for later studies.

SEM images were used to study surface morphologies before and after polyGMA grafting. Fig. 2 shows SEM images of blank PBT and PBT after polyGMA grafting at different BP to GMA ratios. The grafting conditions were 15 min grafting and GMA concentration at 2.0 M with ratios of BP to GMA of 0.5:100, 1:100 and 2:100. Row A contains SEM images of blank PBT. Rows B, C, and D contain SEM images of PBT-GMA with grafting conditions at BP:GMA=0.5:100, 1:100 and 2:100, respectively. Row E contains SEM images of fiber cross sections from the sample with grafting condition at BP:GMA=2:100 (shown in Row D). Images in the first and second column come from two sides of the nonwoven membranes, and images in the last column have a higher magnification. Similar fiber morphologies were observed on both sides of the nonwoven as seen by comparing the images in the first and second column. From the images shown in row A, it can be observed that the fibers in the blank PBT membrane have a smooth surface. After the nonwoven was subjected to grafting using a BP to GMA ratio of 0.5:100 (images in row B), the fibers appear to have a much rougher surface. When the ratios of BP to GMA increase to 1:100 (images in row C), the grafting layer on the

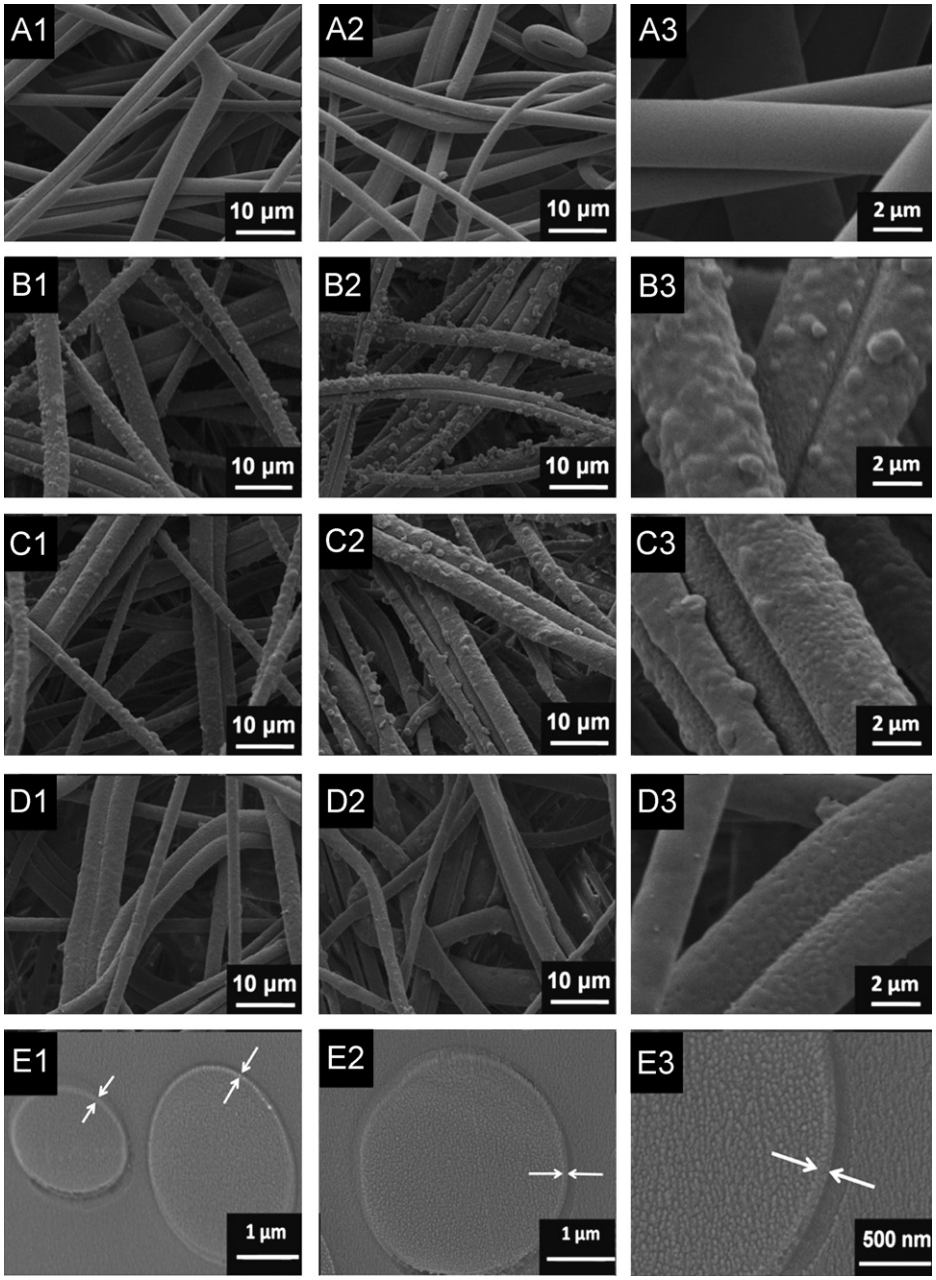

Fig. 2. SEM images of (A) blank PBT surface; (B) PBT-GMA surface after GMA grafting with BP:GMA=0.5:100; (C) PBT-GMA surface after GMA grafting with BP:GMA=1:100; (D) PBT-GMA surface after GMA grafting with BP:GMA=2:100; (E) cross section of fibers after GMA grafting with BP:GMA=2:100. (1) stands for images of the front side of the samples, (2) stands for images of the back side of the samples, (3) stands for images with a higher magnification. The grafting time is 15 min and GMA concentration is 2.0 M.

nonwoven fibers becomes smoother. When the ratio of BP to GMA increases to 2:100 (images in row D), a more uniform grafting layer was achieved. When the BP to GMA ratio is low ($\leq$0.5:100), small amounts of BP can access the fiber surface to create free radicals on the fiber surface under UV light. As a result, the radical density on the fiber surface is low and the consequently grafted layer is not very uniform at low chain densities. When the BP concentration increases, more BP molecules can reach the fiber surface to create more radical sites, which will initiate high density of growing chains and more uniform grafting layer. Combining results from Figs. 1 and 2, the highest degree of GMA grafting was achieved at a BP to GMA ratio of 1:100 and a more uniform grafting layer was achieved at a BP to GMA ratio at 2:100. The ratio of BP to GMA of 2:100 was used for later studies because a more uniform grafted layer is likely to result in lower nonspecific protein binding.

Row E shows the cross section from some fibers in the membranes shown in row D. The images show the cross section of a cylindrical fiber with a uniform circular grafted layer around the PBT fiber with a layer thickness of approximately 100 nm according to the SEM image scale. With the assumption that the grafted layer has approximately the same density as that of PBT bulk polymer, the degree of GMA grafting, or fractional weight gain due to grafting, is related to the grafted layer thickness and the fiber radius, by the following expression,

$$\text{The degree of GMA grafting} \approx \frac{(R+\delta)^2 - R^2}{R^2} \quad (4)$$

Here δ is grafting layer thickness, R is original fiber radius.

According to Eq. (4), the grafted layer thickness δ should be around 75 nm given the degree of GMA grafting to be 10% and a mean fiber radius R of 1.5 μm. The result of this simple calculation is consistent with the SEM image measurement.

After polyGMA grafting, FTIR was used to confirm the surface chemistry of the membrane. Fig. 3 shows the FTIR spectra of (A) blank PBT, (B) PBT sample exposed to UV light in 1-butanol without monomer and photoinitiator for 15 min, (C) PBT with GMA grafting using 2.0 M GMA in 1-butanol with a ratio of BP:GMA=2:100 for 15 min, and (D) pure polyGMA synthesized from solution polymerization with benzoyl peroxide as initiator. Both sides of the nonwoven samples were analyzed and at least three points were recorded for each side of every sample. Fig. 3 shows representative spectra for each one of the materials tested.

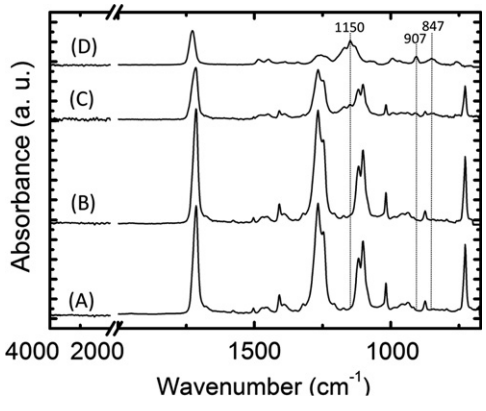

Fig. 3. FTIR spectra of (A) blank PBT; (B) PBT exposed to UV in 1-butanol without GMA and BP for 15 min (C) PBT-GMA after GMA grafting in 2.0 M GMA with BP:GMA=2:100 for 15 min; (D) polyGMA synthesized from solution polymerization. In spectra (A) and (B), Peak at 1714 cm^{-1} corresponds to carbonyl group, peaks at 1267 cm^{-1} and 1102 cm^{-1} correspond to ester group from PBT, peak at 727 cm^{-1} corresponds to benzene ring. In spectra (C) and (D), new peaks at 1150 cm^{-1} correspond to ester group from polyGMA, peaks at 907 cm^{-1} and 847 cm^{-1} correspond to epoxy group.

Comparing the spectrum of blank PBT with the PBT sample subjected to grafting conditions minus GMA and BP (spectra A and B, respectively, in Fig. 3), it can be observed that they are identical, indicating that UV light and 1-butanol alone did not change the chemical composition of the membrane. Through the comparison of the spectra of the blank PBT and the PBT-GMA membranes (spectra A and C in Fig. 3), the spectrum of the PBT-GMA shows the characteristic ester peaks (at 1150 cm^{-1}) and epoxy group peaks (907 cm^{-1} and 847 cm^{-1}) from grafted polyGMA. Those peaks can also be observed in the pure polyGMA (spectrum D, Fig. 3), which indicates polyGMA was formed on nonwoven membrane after surface modification. FTIR spectra on each side of the polyGMA grafted nonwoven sample are very similar (data not shown here), which suggests that this UV-grafting surface modification technique can modify all the fibers in a nonwoven membrane at used conditions by exposing only one side of the PBT nonwoven membrane to UV light. This result is consistent with SEM analysis results by comparing images from both sides of the membrane. SEM images and FTIR spectra prove UV penetration is not a problem in grafting polyGMA on the PBT nonwoven because both sides of the nonwoven membrane have a similar grafting result. There are references in the literature indicating that UV grafting is restricted to thin films because of the relatively poor UV penetration of many samples [46]. However, the fact that nonwoven fabrics such as the PBT used in this study have relatively large pore sizes in the range of several micrometers, high porosity at 87% and fabric thickness of only around 300 μm makes the UV grafting method suitable for surface grafting around fibers on this material.

3.2. Grafting kinetics

The effect of GMA grafting time was studied with GMA concentrations of 1.5 M and 2.0 M and a constant ratio of BP to GMA of 2:100. Fig. 4 shows the relationship between the degree of GMA grafting and grafting time at these two GMA concentrations. When the grafting time is greater than 5 min, there is a linear relationship between the degree of GMA grafting and grafting time for both 1.5 M and 2.0 M GMA with the highest GMA grafting being approximately 23% and 33% after 30 min. The degree of GMA grafting is higher with 2.0 M GMA solution than with 1.5 M, which is consistent with our earlier result shown in Fig. 1. Because of these results, all samples produced for further study were made using the optimized grafting conditions of 2.0 M GMA solution with a BP:GMA=2:100. The reason there is almost no grafting when the grafting time is less than 5 min is due to the presence of oxygen in the grafting solution that suppresses grafting during this initial phase. The results of Fig. 4 indicate

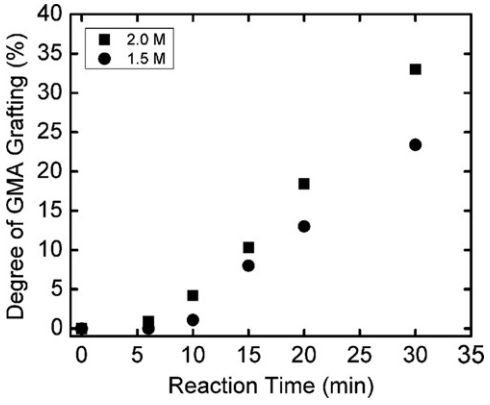

Fig. 4. Degree of GMA grafting with grafting time (GMA concentration is 1.5 M (●) and 2.0 M (■), and BP:GMA=2:100).

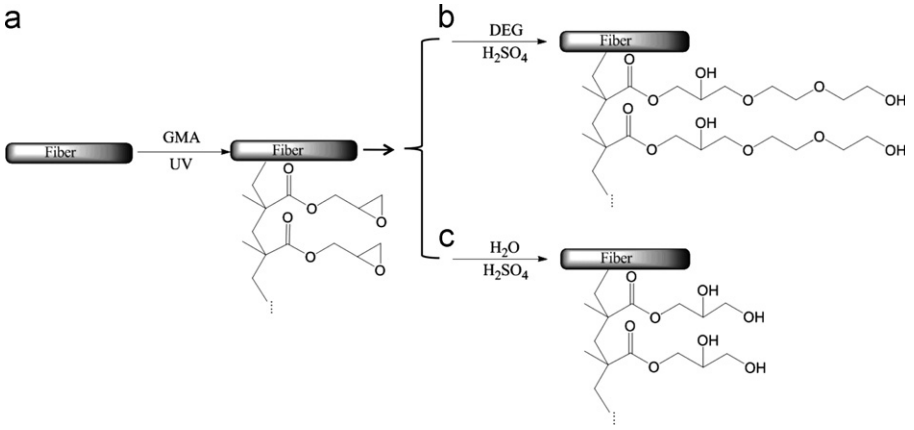

Fig. 5. Schematic diagram of polyGMA grafting and hydrophilization of PBT nonwoven. The first step is UV-induced grafting to achieve (a) PBT-GMA, the second step is diethylene glycol (DEG) or diol attachment to achieve (b) PBT-GMA-DEG or (c) PBT-GMA-diol.

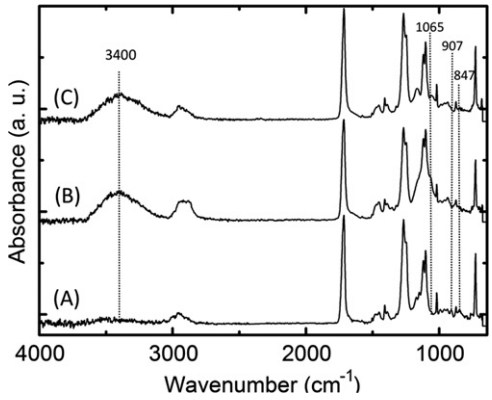

Fig. 6. FTIR spectra (A) PBT-GMA; (B) PBT-GMA-DEG; (C) PBT-GMA-diol. GMA grafting was in 2.0 M GMA with a BP:GMA=2:100 for 15 min. In spectra (A), peak at 1714 cm^{-1} corresponds to carbonyl group, peaks at 1267 cm^{-1} and 1102 cm^{-1} correspond to ester group, peak at 727 cm^{-1} corresponds to benzene ring, peaks at 907 cm^{-1} and 847 cm^{-1} correspond to epoxy group. In spectrum (B), new hydroxyl group peak centered at 3400 cm^{-1} and new ether group peak at 1065 cm^{-1} appear, and epoxy group peaks at 847 cm^{-1} and 907 cm^{-1} disappear. In spectrum (C), new hydroxyl group peak appears and epoxy group peaks disappear.

that it is possible to use the grafting time as an effective way of controlling the grafted layer thickness.

3.3. Hydrophilization by DEG attachment or hydrolysis reaction

After the PBT nonwoven was grafted with one layer of polyGMA, DEG or diol groups were introduced to the fiber surface to hydrophilize nonwoven fibers by reacting the epoxy groups from polyGMA with DEG or water. The reaction processes used are shown in Fig. 5. The FTIR spectra of the PBT-GMA, PBT-GMA-DEG and PBT-GMA-diol membranes are shown in Fig. 6 as spectrum (A), (B) and (C), respectively. The characteristic hydroxyl group peak (around 3400 cm^{-1}) and ether group peak (1065 cm^{-1}) found in spectrum (B) confirm the successful attachment of DEG. The hydroxyl group peak was also found in spectrum (C), demonstrating the successful GMA hydrolysis. Epoxy group peaks at 907 cm^{-1} and 847 cm^{-1} disappeared after DEG attachment or epoxy hydrolysis, indicating that the epoxy groups on the fiber surface were converted successfully.

3.4. Membrane characterization

Contact angle measurements provide a useful technique to test whether the membrane surface changed from hydrophobic to hydrophilic. In this work, contact angle measurements show the water contact angle of blank PBT without any treatment to be 108 degree. However, the membranes absorbed the water drop immediately after DEG attachment or hydrolysis treatment (PBT-GMA-DEG or PBT-GMA-diol membrane). The hydrostatic penetration pressure of one layer of blank PBT was found to 2450 Pa and it decreased to 0 Pa for the PBT-GMA-DEG or PBT-GMA-diol membrane. These results demonstrate the hydrophobic PBT nonwoven membrane was modified to form a completely hydrophilic membrane after either DEG attachment or epoxy group hydrolysis.

Table 2
Membrane properties before and after hydrophilization.

	Blank PBT	PBT-GMA-DEG	PBT-GMA-diol
Contact angle (degree)	108	0	0
Hydrostatic penetration pressure (Pa)	2450	0	0
Mean flow pore size (μm)	8 ± 0.5	7.4 ± 0.7	7.6 ± 0.5

The original PBT membrane had a mean flow pore size of 8 ± 0.5 μm, the DEG modified membrane showed a mean flow pore size of 7.4 ± 0.7 μm and the diol-modified membrane had a mean flow pore size of 7.6 ± 0.5 μm. The average mean flow pore size decreased about 0.5 μm after polyGMA grafting and DEG or diol attachment to the fiber surface. These data are summarized in Table 2.

The surface morphologies of the nonwoven membranes before and after surface treatments–including blank PBT, PBT-GMA, PBT-GMA-DEG and PBT-GMA-diol, were studied using SEM images. For every sample two sides were analyzed with at least two spots on each side. The representative images are shown in Fig. 7 with image (A) for blank PBT, (B) for PBT-GMA, (C) for PBT-GMA-DEG and (D) for PBT-GMA-diol. Comparing these four images, the pore structure between fibers is very similar. Combining the results in Fig. 7 and Table 2, it can be concluded that the porous structure of the nonwoven membrane is essentially maintained after surface treatments, with the exception of a slightly smaller mean flow pore size due to the grafted layer on the fibers.

Flow permeability measurements were used to characterize the blank PBT, PBT-GMA-DEG and PBT-GMA-diol. A column filled with 0.65 g of nonwoven fabrics at a fixed column height of 0.5 cm with disc diameter of 2.2 cm was used for this purpose, and the pressure differences between the column inlet and outlet at different flow rates were measured. The packing density of the membrane column is 0.34 g/ml, which corresponds to packed membrane porosity of 74% according to Eq. (2). A schematic

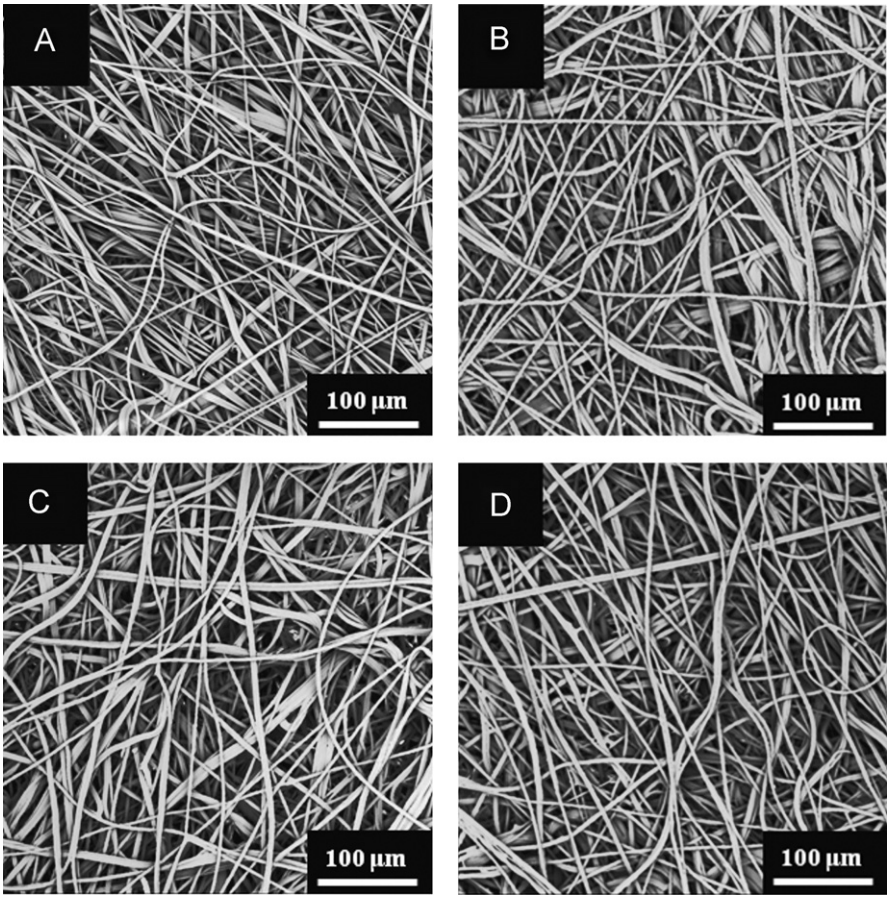

Fig. 7. SEM images of (A) blank PBT, (B) PBT-GMA, (C) BT-GMA-DEG; (D) PBT-GMA-diol. GMA grafting was in 2.0 M GMA with a BP:GMA=2:100 for 15 min, DEG attachment is in DEG with catalytic H_2SO_4 amounts at 50 °C for 8 h and hydrolysis is in 0.1 M H_2SO_4 at 50 °C overnight.

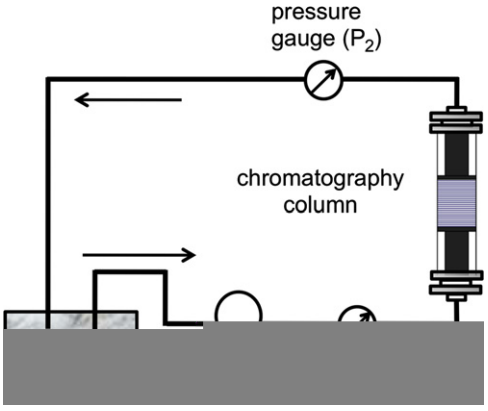

Fig. 8. Schematic diagram of flow permeability measurement. 0.65 g nonwoven membranes were packed in the column at a column height of 0.5 cm.

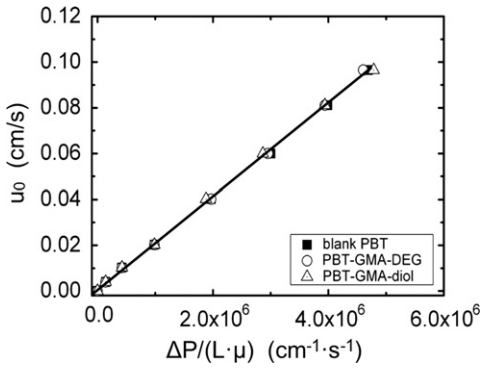

Fig. 9. Superficial flow velocity vs pressure drop data for columns packed with the blank PBT, PBT-GMA-DEG and PBT-GMA-diol nonwoven membranes with 0.65 g packed at a column height of 0.5 cm with diameter of 2.2 cm.

diagram of the flow permeability measurement apparatus is shown in Fig. 8. Experimental results for the pressure difference as a function of superficial velocity are showed in Fig. 9. The pressure differences during flow measured with the PBT-GMA-DEG, PBT-GMA-diol and blank PBT membranes are similar to each other at the same flow rates, again indicating that PBT nonwoven fabrics maintained their porous structure after surface modification. According to Darcy's law (Eq. (5)), the slope in Fig. 9 can be used to calculate the nonwoven membrane flow permeability.

$$u_0 = k \cdot \frac{\Delta p}{\mu \cdot L} \tag{5}$$

Here u_0 is flow rate (cm/s), k is permeability (cm^2), Δp is the pressure difference caused by flow through the membrane (Pa), μ is dynamic viscosity (Pa·s) and L is membrane column length (cm).

The calculated flow permeability for the membrane is $2 \times 10^{-8}\,cm^2$. This value is about 1 order of magnitude higher than the literature value of ion exchange columns packed with resins [47,48]. The primary reason for the higher permeability values for nonwovens is the very high porosity of the packed column of nonwoven membranes (0.74) compared to chromatography columns filled with resin particles (approximately 0.4).

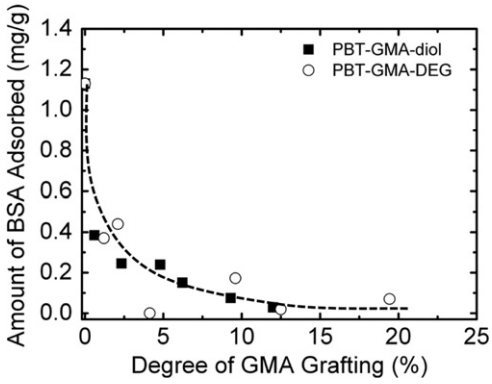

Fig. 10. BSA adsorbed amount on nonwoven membranes with different degrees of GMA grafting followed by DEG and diol attachment. The sample with 0% degree of GMA grafting is blank PBT without any treatment. BSA solution is 0.5 mg/ml in PBS buffer at pH 7.4.

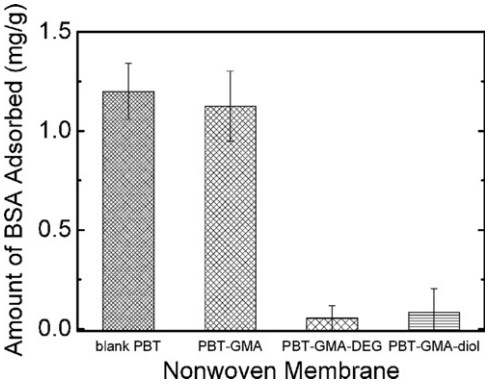

Fig. 11. BSA adsorbed amounts on the membranes of blank PBT, PBT-GMA, PBT-GMA-DEG and PBT-GMA-diol. BSA binding solution is 0.5 mg/ml BSA in PBS buffer at pH 7.4.

3.5. Reduction of nonspecific protein binding

Measurements were taken on the nonspecific protein binding of BSA to PBT-GMA-DEG and PBT-GMA-diol samples with different degrees of GMA grafting in 0.5 mg/ml BSA solutions. Fig. 10 shows static BSA binding amount of these samples with the degrees of GMA grafting ranging from 0 to 20%, which were achieved with different grafting times from 0 to 23 min. Blank PBT was used as the control sample with 0% degree of GMA grafting.

In Fig. 10, it can be seen that the blank PBT has a BSA binding amount of 1.13 mg/g-nonwoven. The amount of bound protein decreases to about 0.4 mg/g-nonwoven when the degree of GMA grafting is 1–2%. The BSA binding amount keeps decreasing and then levels off as the degree of GMA grafting increases. At low degrees of GMA grafting, the polymer grafted layer is thin and the fiber surface is not perfectly covered so that protein molecules can still access some hydrophobic patches on the PBT fiber surface. When the degree of GMA grafting is high, the thicker grafted layer and more uniform surface coverage can prevent protein binding. The BSA adsorption decreases ~80–90% when the degree of GMA grafting is greater than 10%. The degree of GMA grafting was set at 12% from this point on for the following study.

Measurements of the amount of BSA adsorption on the blank PBT, PBT-GMA, PBT-GMA-DEG and PBT-GMA-diol membranes were performed to determine if the resistance to protein adsorption derives from the polyGMA grafted layer or the hydrophilized grafted layer. The BSA binding onto the PBT-GMA membrane is based on protein adsorption instead of the reaction between proteins and epoxy groups because this reaction requires a basic solution [39]. Fig. 11 shows BSA binding results with different surface chemistries. Both blank PBT and PBT-GMA show similar BSA binding abilities at 1.1–1.2 mg/g and both hydrophilized samples of PBT-GMA-DEG and PBT-GMA-diol show similar protein binding amount around 0.1–0.2 mg/g. These results demonstrate that the hydrophilization step by DEG attachment or diol introduction is required to achieve protein binding resistance after polyGMA grafting.

Adsorption isotherms for BSA binding to the various forms of modified PBT nonwoven were measured to determine the effect of protein concentration on nonspecific protein binding. Fig. 12 shows BSA adsorption isotherms on the blank PBT, PBT-GMA-DEG, PBT-GMA-diol, UV/Ozone (UV/O)-treated and plasma-treated PBT nonwovens. The reason UV/O-treated and plasma-treated nonwovens included here is that these two methods are widely used in industry to hydrophilize polymeric nonwoven

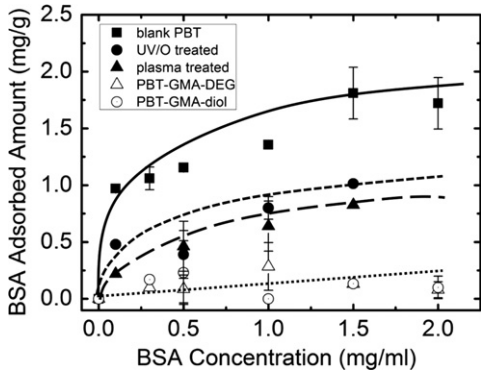

Fig. 12. BSA binding isotherms of the blank PBT, PBT-GMA-DEG, PBT-GMA-diol, UV/O-treated and plasma-treated PBT nonwoven membranes.

fabrics temporarily. From Fig. 12, it can be seen that the maximum BSA binding capacity of blank PBT is around 2 mg/g, which is consistent with a monolayer of BSA binding in the side-on saturation configuration of 2–4 mg/m^2 [49], and recalling that the specific surface area of the PBT nonwoven used in this work is around 0.72 m^2/g. For simple UV/O- and plasma-treated PBT samples, BSA binding capacities were reduced by 40% to 50%, compared to that of blank PBT membrane. For the hydrophilic samples of PBT-GMA-DEG and PBT-GMA-diol, prepared with surface grafting method, BSA binding capacity was reduced around 80–90%. These results demonstrate that the addition of a conformal and uniform polyGMA grafted layer followed by conversion of the epoxy groups into DEG or diol groups is an effective way to decrease nonspecific binding of proteins to the nonwoven membrane. It should also be noted that surface treatments using UV/O or plasma are not permanent and tend to decay in functionality during storage and use.

Just to make sure that the reduction of the nonspecific protein binding was not just an artifact of using BSA as the model protein, additional adsorption measurements were done with other proteins. Lysozyme (from chicken egg white) has a molecular weight 14.3 kDa and an isoelectric point (pI) of 11.35. Beta-casein (from bovine milk) has a molecular weight 24 kDa and pI around 5. Human immunoglobulin G (hIgG) has a molecular weight 150 kDa and pI around 8. Fig. 13 shows the reduced protein binding results for β-casein, hIgG and lysozyme. The protein binding amount of the blank PBT nonwoven for β-casein, hIgG and lysozyme is around 1.6, 2.86, 1.74 mg/g, respectively, which corresponds to 2.2 mg/m^2, 4 mg/m^2 and 2.4 mg/m^2 based on a specific surface area at 0.72 m^2/g of PBT nonwoven membrane. These data are in the right range compared with literature data:

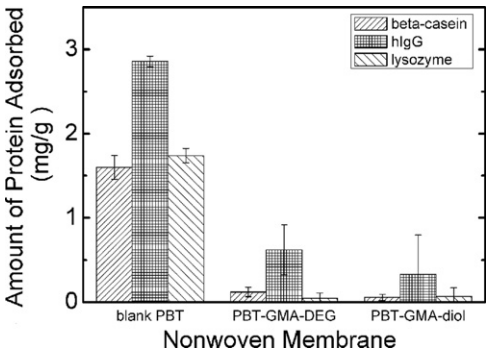

Fig. 13. Amounts of beta-casein, hIgG and lysozyme adsorbed on the membranes of blank PBT, PBT-GMA-DEG and PBT-GMA-diol. Protein solutions are at a concentration of 2 mg/ml in PBS buffer at pH 7.4.

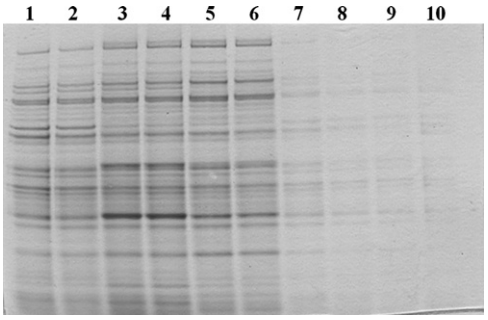

Fig. 14. SDS-PAGE results of eluted proteins from nonwoven membrane packed columns after being challenged with *E. coli* extract. Nonwoven membranes used in lanes 1, 2: original PBT; lanes 3, 4: UV/O-treated PBT; lanes 5, 6: plasma-treated PBT; lanes 7, 8: PBT-GMA-diol; lanes 9, 10: PBT-GMA-DEG.

1.9, 3-4, 2.5 mg/m^2 for β-casein, hIgG and lysozyme respectively for protein adsorption on a hydrophobic surface [50,51]. After surface treatment with GMA grafting and hydrophilization (DEG or diol attachment), the nonspecific protein binding ability was reduced to 0.1–0.2 mg/g for β-casein and lysozyme and 0.4–0.7 mg/g for hIgG, respectively. For these three different proteins with molecular weights ranging from 14 to 150 kDa and pI ranging from 5 to 11.35, the PBT-GMA-DEG or PBT-GMA-diol membranes were able to reduce nonspecific protein binding by approximately 80%.

3.6. Reduction of protein binding in clarified E. coli extract

Even though the reduction of nonspecific protein binding using single protein showed good results, for actual applications it is necessary to investigate the nonspecific adsorption of proteins from more complex mixtures. A clarified *E. coli* extract was exposed to the surface modified PBT nonwovens to quantify the percent reduction in nonspecific adsorption using a more challenging system. The results of these experiments are shown in Fig. 14 in the form of electrophoresis gels of the bound proteins after they had been eluted from the samples.

The same amount of *E. coli* extract (10 ml) was used to challenge columns filled with 20 layers of nonwovens, at the same flow rate. The total protein concentration of the challenge was 5 mg/ml by BCA measurement. After binding and washing, the bound protein was eluted from the column with the same amount of sample buffer. Because the protein binding interactions might be complex and different for the membranes with different surface treatments, sample buffer (containing sodium dodecyl sulfate) and heat were used to denature the bound proteins from the membranes and used directly in the SDS-PAGE analysis.

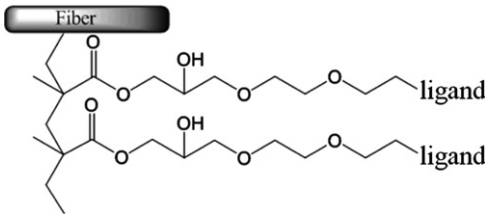

Fig. 15. Grafting layer molecular structure of developed affinity membrane.

In Fig. 14, lanes 1 and 2 have the eluted proteins from columns packed with blank PBT membranes; lanes 3 and 4 are eluted protein from the columns packed with the UV/O-treated PBT membranes; lanes 5 and 6 are eluted protein from the columns packed with the plasma-treated PBT membranes; lanes 7 and 8 are eluted protein from the columns packed with the PBT-GMA-diol membranes; and lanes 9 and 10 are eluted protein from the columns packed with the PBT-GMA-DEG membranes. The intensity of the signal in the protein bands on the gel is roughly proportional to the amount of bound protein. From the gel signal, the binding ability of some proteins to UV/O-treated PBT and plasma-treated PBT membranes decreased and the ability of other proteins to bind actually increased, compared with nonspecific protein binding to the blank PBT membrane. But for membranes treated with polyGMA grafting and diol conversion or DEG attachment, the total protein binding was reduced dramatically and uniformly for all of the proteins in the mixture. By analyzing the optical density of the major protein bands on the gel with the software program Image J, the total amount of protein binding was reduced by about 80% and 85% for the PBT-GMA-diol membrane and the PBT-GMA-DEG membrane respectively. These results show that surface treatment of polyGMA grafting and DEG or diol attachment endows the membrane with a significant nonspecific protein adsorption resistance even for a very complex system such as *E. coli* extract.

There are numerous indications in the literature that the benchmark used to prevent protein adsorption to the surfaces is poly (ethylene glycol) (PEG) [52]. From the results shown above, grafted polyGMA layer and hydrophilization of the epoxy groups using DEG or diol functionalities were shown to decrease nonspecific protein binding to the membranes. The level of nonspecific binding exhibited by the treated membranes seems to be sufficiently low to enable their use for microfiltration of particulate materials from complex biological fluids.

3.7. Target prion protein capture from hamster brain homogenate

Having demonstrated that the surface modifications described previously can significantly reduce nonspecific protein binding, attention was turned to performing an actual separation using a specific affinity ligand from a complex mixture, and the removal of prion protein from normal hamster brain homogenate was selected.

Infectious prion proteins are contaminants in blood, blood products and other fluids and tissues that can lead to the transmission of variant Creutzfeldt-Jakob Disease (vCJD) in humans. Our research group has identified a small ligand that is able to remove the prion protein selectively from blood, plasma and diseased brain tissue homogenates [42,43]. This ligand was attached to the PBT-GMA-DEG membrane, which previously was found to have low levels of nonspecific protein binding for a complex mixture (shown in Fig. 14). A schematic of the grafted layer molecular structure of this affinity membrane is shown in Fig. 15. The ability of this affinity membrane to capture prion

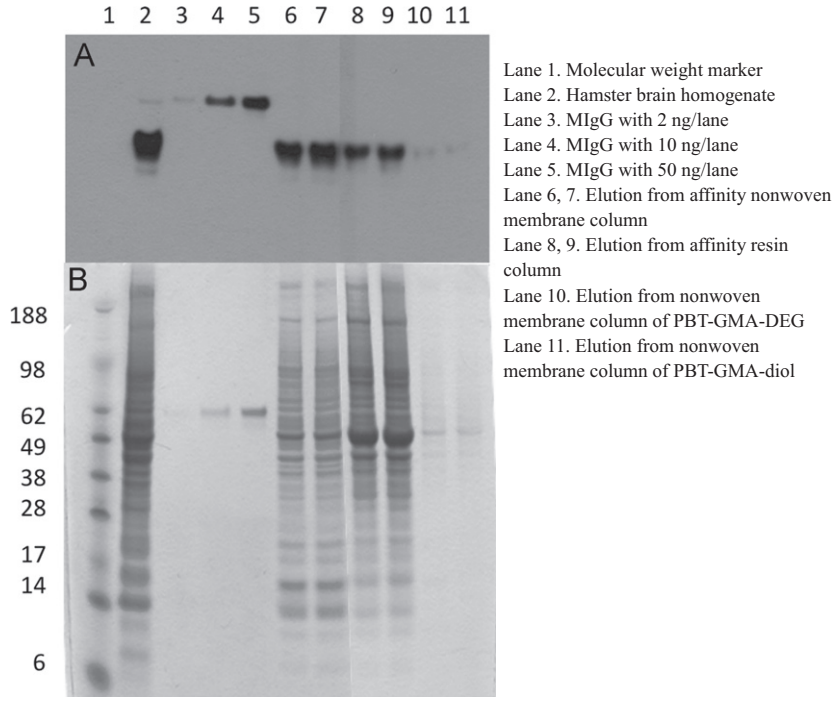

Fig. 16. Western blot (A) and SDS-PAGE (B) results of the eluted samples from different membranes or resins packed columns after being challenged with hamster brain homogenate. Lane 1: molecular weight marker; lane 2: hamster brain homogenate; lane 3: mouse IgG (2 ng); lane 4: mouse IgG (10 ng); lane 5: mouse IgG (50 ng); lanes 6 and 7: elution fraction from affinity nonwoven membrane column; lanes 8 and 9: elution fraction from affinity resin column; lane 10: elution from nonwoven membrane column of PBT-GMA-DEG; lane 11: elution from nonwoven membrane column of PBT-GMA-diol.

protein selectively from hamster brain homogenate was tested experimentally.

A solution spiked with hamster brain homogenate (HaBH) containing the normal form of the prion protein was used in this experiment. The same amount of HaBH solution (6 ml) was exposed to the samples of the various forms of modified membrane (the prion affinity membrane, the PBT-GMA-DEG membrane and the PBT-GMA-diol membrane) and an affinity resin containing the same ligand (control). The membranes and resin were packed into PIKSI columns and the challenge solution was passed at the same flow rate through each column. The elution of the proteins bound to the membranes and the resins was done in exactly the same way for all samples. After elution, the same amount of elution solution was injected into the SDS-PAGE gel for analysis. The results for prion protein capture and total protein binding from HaBH solution were determined by Western blot and SDS-PAGE, respectively, and are shown in Fig. 16. The Western blot uses an antibody that is specific to the prion protein and as a result the intensity of the bands seen on the gels correlate with the amount of prion protein captured by the membrane or resin.

In both Western blot analysis (Fig. 16A) and SDS-PAGE analysis (Fig. 16B), lane 1 shows the molecular weight markers. Lane 2 is the hamster brain homogenate load. Lanes 3 to 5 show the molecular weight standards of mouse immunoglobulin G (MIgG) with low, medium and high amount by 2 ng/lane, 10 ng/lane and 50 ng/lane, respectively, to illustrate the relation between the band intensity on the gels and protein amounts. For both SDS-PAGE and Western blot, the intensity of the bands on the gels is proportional to the amounts of proteins. Lane 6 and lane 7 show duplicate elutions from the affinity nonwoven membranes. Lanes 8 and 9 show duplicate elutions from the affinity resins, which is used as a positive control. Lanes 10 and 11 are the proteins eluted from the nonwoven membrane PBT-GMA-DEG and PBT-GMA-diol, respectively.

In the Western blot shown (Fig. 16A), the signals in lanes 2, 6, 7, 8 and 9 correspond to prion protein and the signals in lanes 3, 4 and 5 correspond to the MIgG, which demonstrates the relation between gel bands intensity and protein amount on the Western blot image. From this image, the affinity membranes (in lanes 6 and 7) show the ability to capture prion protein, which is higher than the prion protein capture ability of the affinity control resin (in lanes 8 and 9) as indicated by the gel band intensity comparison.

SDS-PAGE shows the total protein profile eluted from the membranes and control resin. According to SDS-PAGE results, the nonwoven membranes of PBT-GMA-DEG and PBT-GMA-diol show a very low nonspecific protein binding for a HaBH solution (in lanes 10 and 11, respectively). This is consistent with the previous result that these two hydrophilized membranes show a lower nonspecific protein binding ability for *E.coli* extract compared with unmodified PBT membrane (as shown in Fig. 14). The affinity nonwoven membranes (in lanes 6 and 7) demonstrate a binding pattern for proteins that is similar to the control affinity resin (lanes 8 and 9), but with a lower total protein binding. As a result, the high signal on a Western blot that is accompanied by a low signal on the SDS-PAGE gels indicates that the developed affinity membranes exhibit specific prion protein binding capabilities with low nonspecific protein binding.

The results from this experiment indicate that it is possible to develop nonwoven membranes that have very low nonspecific protein binding capacity, but are able to bind to targeted proteins specifically via the use of specific ligands for these target molecules. The application of this concept can be easily extended to the development of other affinity membranes, for which the elimination of nonspecific protein binding is highly desired.

4. Conclusions

A conformal and uniform polyGMA grafted layer on 3-dimensional PBT nonwoven fabrics was achieved by UV-induced grafting polymerization under optimized grafting

conditions. FTIR spectra and SEM images were used to prove chemical and morphology changes after surface grafting and these results show both sides of the nonwoven have undergone similar levels of grafting. GMA concentration and BP concentration have a big effect on the degree of grafting and grafting morphology. The degree of GMA grafting increases with GMA concentration and increases first and then decreases with the ratios of BP to GMA. The degree of GMA grafting has the highest

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