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Fabrication of electrospun PVDF nanofiber membrane for Western blot with high sensitivity

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ABSTRACT

A microphase separation-based porous polyvinylidene fluoride (PVDF) membrane has been generally used for immobilizing proteins in a Western blotting procedure. The conventional PVDF membrane is extremely hydrophobic and will not wet in aqueous solutions unless pre-wetted with methanol. In addition, long-term immersion of the PVDF membrane in methanol deteriorates both its protein binding capacity and mechanical strength. This study showed that a PVDF membrane consisting of long nanofibers, which were prepared by electrospinning and subsequent calendering, was mechanically strong with uniform and opened pores. This membrane exhibited high sensitivity, low background and high binding capacity for the detection of protein bands without a methanol pre-wet step.

1. Introduction

Western blotting or immunoblotting is used widely in the field of life sciences and biochemistry [1–4]. Polyacrylamide gel electrophoresis (PAGE) is used as a matrix to separate the proteins according to the molecular weights or three dimensional (3D) structures. The separated proteins need to be transferred to a membrane, such as nitrocellulose (NC) or polyvinylidene fluoride (PVDF), from the gel matrix because they are not easy to access with molecular probes and PAGE is very difficult to handle in the subsequent detection steps. During the transfer step, the gel electrophoresis pattern of the proteins is maintained. The driving forces of protein transfer are simple diffusion [5], capillary action [6], vacuum-associated solvent flow [7] and electrophoretic transfer [8]. Among them, electrophoretic transfer is by far the most commonly used transfer method because of its high speed and complete transfer [1]. Eventually, the proteins are immobilized on a thin surface layer of the membrane and can be detected by antibodies that recognize the target protein.

The membranes made of NC or PVDF, which are produced by microphase separation, are commercially available for Western blotting. The initially used NC membranes are mechanically weak to repeated probing. Therefore, PVDF membranes are mainly used for their good retention capacity and excellent mechanical strength under extreme conditions (i.e. in organic solvents or under acidic or basic conditions).

Conventional PVDF membranes are extremely hydrophobic and will not wet in aqueous solutions unless pre-wetted with methanol. On the other hand, long-term immersion of the PVDF membranes in methanol can deteriorate both its protein binding capacity and mechanical strength to some degree. Therefore, methanol pre-wetting of the PVDF membrane is a cumbersome process. Herein, this study demonstrated that the electrospun PVDF nanofiber membrane is mechanically strong and exhibits high binding capacity for the detection of protein bands or spots, without a methanol pre-wet step. Such enhanced protein blotting ability can be explained by a large accessible surface area.

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2. Experimental details

2.1. PVDF nanofiber membrane preparation

The PVDF polymer (Kynar®761, molecular weight = 441,000; ARKEMA, Calvert City, KY, USA) was dissolved in dimethylacetamide (DMAc; Aldrich, Milwaukee, WI, USA) to form a 20 wt% solution. The polymer solution was transferred to a spin pack containing spinnerets (capillary tip = 0.5 mm diameter). Using a typical apparatus (Amotech Co., Kimpo, Korea), electrospinning was carried out by applying a high positive voltage (100 kV) to the polymer solution via the spinneret tip [9,10]. The electrospun fibers were collected as a thin web on a current collector plate with a suction collector. The electrospun nanofiber web was then passed under rollers at 140 °C to fabricate a dense PVDF nanofiber membrane.

The morphology and structure of the PVDF nanofiber membrane were characterized by field emission scanning electron microscopy (FESEM, JEOL JSM-6335F, 5 kV; JEOL Ltd., Tokyo, Japan). The porosity of the membrane was measured using the following Eq. (a):

$$\text{Porosity}\,(\%) = \frac{\rho_0 - \rho}{\rho_0} \times 100 \qquad \text{(a)}$$

where ρ_0 is the density of PVDF (1.76 g/cm^3) and ρ is the measured density value.

The tensile strength of the membranes were measured using a micro-strain tester (MST-I, Shimadzu, Tokyo, Japan).

2.2. Cell culture and total protein preparation

KB cells, an oral epithelial carcinoma cell line, were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The KB cells were grown in MEM medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen) at 37 °C in a humidified atmosphere containing 5% CO_2.

For harvesting, the cells were washed twice with PBS. The cells were lysed in RIPA buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.25% sodium deoxycholate, 0.1% Nonidet P-40, 0.1% SDS, 50 mM NaF, 0.5 mM phenylmethylsulfonyl fluoride, 10 mM Na_3VO_4 and Complete Mini protease inhibitor cocktail [Roche Diagnostics, Mannheim, Germany]) for 30 min on ice. After centrifugation, the supernatant was transferred to a new tube. The protein concentration was measured by the Bradford method using the Bio-Rad Protein Assay Dye Reagent Concentration (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol.

2.3. Western blotting

The proper amount of protein, ranging from 100 to 0.5 μg, from the KB cell lysate was electrophoresed on SDS–PAGE and transferred to the commercial microphase separation-based porous PVDF membrane and PVDF nanofiber membrane. It is well known the conventional PVDF membrane is extremely hydrophobic and will not wet in aqueous solutions unless pre-wetted with methanol. To investigate if the nanofiber PVDF membrane can be useful in Western blots without a methanol pre-wet step two groups were tested: in first group, two types of membranes were immersed in methanol for 3 min before equilibrated in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3) and in the second group, two type membranes were equilibrated directly in transfer buffer without a methanol pre-wet step. The transfer of proteins to the membrane was carried out using a Mini-gel transfer kit (XCell SureLock™ Mini-Cell Electrophoresis System, Invitrogen) at 40 V for 1 h. The transfer was carried out by placing the transfer kit on ice to suppress heat generation. Immediately after transfer, the membrane was detached from the kit and washed with a washing buffer (TBST, 0.1% Tween-20, 50 mM Tris–HCl pH 7.5, 150 mM NaCl). The membranes were blocked for 1 h with 5% milk at room temperature and incubated at 4 °C for with each primary antibody, anti-β-actin (sc-47778, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-phospho-eIF4G (#2441, Cell Signaling Technology, Beverly, MA, USA), anti-REST (07-579, Upstate Biotechnology, Waltham. MA, USA), anti-c-Myc (sc-56634, Santa Cruz Biotechnology), anti-cleaved caspase-9 (#9501, Cell Signaling Technology), anti-cleaved caspase-7 (#9491, Cell Signaling Technology), ant-cytochrome C (sc-13156, Santa Cruz Biotechnology). After washing the membrane three times with TBST (0.1% Tween-20, 50 mM Tris–HCl pH 7.5, 150 mM NaCl), the membranes were incubated with the secondary antibodies at room temperature for 2 h and then washed three times with TBST. The membranes were detected using the WEST-SAVE Up™ luminol-based ECL reagent (ABFrontier, Seoul, Korea) and exposed to KODAK BioMax Light Film (Carestream health Inc., Rochester, NY, USA).

3. Results and discussion

3.1. Characterization of PVDF nanofiber membrane

The PVDF polymers were electrospun successfully into a sheet (width = 1.7 m) by applying a voltage of 100 kV and a tip-to-collector distance of 50 cm. The white membrane was prepared in the form of a roll (Fig. 1a) by passing it through the calender at 140 °C. The purpose of the calender was to make a nanofiber web with a smooth and clean surface by reducing the fluffy nature of the electrospun nanofiber web. The thickness of the electrospun nanofiber web was decreased by up to 40% after the calendering process. Eventually, the PVDF nanofiber membrane was cut into a size of 6 cm × 8 cm for Western blotting (see insert in Fig. 1b). The white membrane was very thin (80 μm) (see inset in Fig. 1d) and consisted of long and continuous PVDF nanofibers with a diameter of 300 nm (Fig. 1c). Considering the use of the PVDF nanofiber membrane in Western blotting, the pore size and porosity of the membrane are important factors for determining the binding capacity of a protein. The pore size corresponds to the diameter of the largest particle that can pass through the membrane structure, whereas the porosity indicates the portion of voids over the total volume as a percentage between 0 and 100%. The commercial PVDF membrane, which is synthesized by microphase separation, exhibits a non-uniform pore diameter and a closed pore structure (Fig. 2a and b); its average pore size is ~0.45 μm but its pores are distributed widely (Fig. 3a). On the other hand, the porosity of the PVDF nanofiber membrane was found to be ~73.3% and its pore size distribution was extremely narrow and centered at 0.3 μm (Fig. 3b). As shown in Fig. 1c, the pore corresponds to empty spaces located between randomly stacked long nanofibers in the form of a thin membrane. In other words, both the porosity and pore size of PVDF nanofiber membrane can be tailored by changing the diameter of the PVDF nanofiber, density of the membrane and the alignment of PVDF nanofibers.

The 3D pore structure of the PVDF nanofiber membrane is expected to be stable and flexible, because fibers are characterized by their flexibility and high aspect ratio (above 100). To confirm this assumption, mechanical experiments were carried using a micro-strain tester. From the typical stress-strain curves (Fig. 4), the tensile strength of the nanofiber membrane was three times higher than the commercial membrane, even though a large decrease in elongation was observed. The possible explanations for this data are (1) the largely enhanced mechanical strength of the nano-sized individual fiber and (2) the fiber-to-fiber contact density.