Journal of Membrane Science 323 (2008) 77–84

Contents lists available at ScienceDirect

Journal of Membrane Science

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

Biofouling-resistance expanded poly(tetrafluoroethylene) membrane with a hydrogel-like layer of surface-immobilized poly(ethylene glycol) methacrylate for human plasma protein repulsions

Yung Chang*, Ting-Yun Cheng, Yu-Ju Shih, Kueir-Rarn Lee, Juin-Yih Lai

R&D Center for Membrane Technology and Department of Chemical Engineering, Chung Yuan University, Jhong-Li, Taoyuan 320, Taiwan

ARTICLE INFO

Article history:
Received 1 March 2008
Received in revised form 29 May 2008
Accepted 5 June 2008
Available online 19 June 2008

Keywords:
Poly(tetrafluoroethylene)
Poly(ethylene glycol) methacrylate
Surface-activated plasma treatment
Graft copolymerization
Plasma proteins
Biofouling-resistance
Blood compatibility

ABSTRACT

In general, it is a challenge to control the highly polar material grafting from the chemically inert Teflon-based membrane surface. This work describes the surface modification and characterization of expanded poly(tetrafluoroethylene) (ePTFE) membranes grafted with poly(ethylene glycol) methacrylate (PEGMA) macromonomer via surface-activated plasma treatment and thermally induced graft copolymerization. The chemical composition and microstructure of the surface-modified ePTFE membranes were characterized by Fourier transform infrared spectroscopy (FT-IR), contact angle, and bio-atomic force microscopy (bio-AFM) measurements. Biofouling property of the modified membranes was evaluated by the measurements of the plasma protein (γ-globulin, fibrinogen, or albumin) adsorption determined using an enzyme-linked immunosorbent assay (ELISA). In general, the hydrophilicity of the surface of ePTFE membranes increases with increasing the grafting degree of the copolymerized PEGMA. The highly hydrated PEGMA chain on the resulting ePTFE membranes was found to form a surface hydrogel-like layer with regulated coverage in aqueous state, which can be controlled by the content of PEGMA macromonomer in the reaction solution. The relative protein adsorption was effectively reduced with increasing capacity of the hydration for the PEGMA chain grafted on the ePTFE membrane surface. From both results of protein adsorption and platelet adhesion test in vitro, it is concluded that the PEGMA-grafted hydrophilic ePTFE membranes could provide good biofouling resistance to substantially reduce plasma protein and blood platelet fouling on the membrane surface in human body temperature.

1. Introduction

Poly(tetrafluoroethylene) (PTFE) is one of the most popular biomaterials for various types of biomedical devices due to its outstanding properties, including excellent chemical resistance, good thermal stability, and mechanical properties [1–4]. However, poor wettability or the hydrophobic nature of the PTFE surface usually limits its processing properties and applications, especially in biotechnology [5–7]. One of the most important requirements for PTFE membranes in biomedical applications is to reduce the non-specific adsorption of bimolecular when living systems encounter hydrophobic membrane surfaces. It is generally acknowledged that hydrophilic surfaces are more likely to reduce protein adsorption [4]. Therefore, PTFE membranes with hydrophilic surface could extend their range of applications, particularly in bioseparation or bioreactors.

Several strategies have been adopted to make PTFE surface hydrophilic: energetic treatments via plasma or ion-beam, pre-adsorption of proteins, dip-coating with a protective hydrophilic polymer, and surface grafting of hydrophilic segments [2,3,6–13]. Surface grafting is an effective approach to incorporate specific functionalities in existing or commercial PTFE membranes through proper molecular design, while retaining their bulk properties and pore structures. For improving the hydrophilicity and biocompatibility of PTFE membranes, ester group in poly(ethylene glycol) (PEG)-based material is the ideal choice of surface functional moiety with anti-fouling characteristics. PEG-based materials are the most commonly used anti-fouling materials for resisting protein adsorption [14,15]. For the general modification of PTFE membranes via surface graft copolymerization, reactive sites or groups on the membrane surface have to be introduced first by gas plasma, ozone, or UV treatment [8,11,13,16,17]. Then, covalent immobilization of PEG-based species can be achieved with their monomers or macromonomers in solutions. Although hydrophilic PTFE membranes grafted with PEG-based species have been investigated, it is still unclear in our knowledge from the limited literature about the

* Corresponding author. Tel.: +886 3 265 4190; fax: +886 3 265 4198.
E-mail address: ychang@cycu.edu.tw (Y. Chang).

0376-7388/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.memsci.2008.06.023

detailed characteristics of their human plasma protein adsorption and platelet activation as PTFE membrane surface contacts with human blood.

Here we report a simple one-pot preparation approach to introduce PEG-based segments on expanded poly(tetrafluoroethylene) (ePTFE) membrane surface and evaluate its protein fouling property and blood compatibility in detail. In the present work, hydrogen (H_2) plasma-induced graft polymerization of poly(ethylene glycol) methacrylate (PEGMA) on ePTFE membrane surface was first carried out by H_2 plasma pre-treatment and followed by thermal grafting copolymerization in situ. The degree of PEGMA grafting on ePTFE membrane surface was controlled by different ratings of plasma power and concentrations of macromonomer in the reaction solution. The chemical composition and surface microstructure of the PEGMA-modified ePTFE membranes were characterized by contact angle, Fourier transform infrared spectroscopy (FT-IR), and bio-atomic force microscopy (bio-AFM). The amount of adsorbed plasma proteins was determined by an enzyme-linked immunosorbent assay (ELISA) in evaluating blood compatibility of the modified ePTFE membranes. The surface morphology of the modified ePTFE membranes and the biofouling property of the platelet adhesion were studied by scanning electron microscopy (SEM). This work demonstrates the correlation between the degree of membrane surface hydration and the adsorption of plasma protein from platelet poor plasma solution on virgin and PEGMA-grafted ePTFE membranes.

2. Materials and methods

2.1. Materials

ePTFE microporous membranes with an average pore size of 0.24 μm, a thickness of about 500 μm, and a size of 6 cm^2 were purchased from YMT Co., Taiwan and were used as received. PEGMA macromonomer with a molecular weight of about 526 and an average number of ethylene glycol units of about ten were purchased from Aldrich. Deionized (DI) water was used as solvent for the plasma treatment and thermal graft copolymerization. Phosphate buffer saline (PBS) was purchased from Sigma.

2.2. Surface copolymerization

ePTFE membranes, which are commercially available, were obtained from YMT Co. A schematic illustration of surface modification process is shown in Fig. 1. The commercial ePTFE membrane of about 500 μm thickness was pretreated with tunable power of H_2 plasma generated from a custom-built plasma reactor. After the H_2 plasma treatment, the ePTFE membrane was allowed to react in situ at 80 °C for 6 h with PEGMA macromonomer with concentration adjusted from 5 to 25 wt.% to achieve the desired grafting degree of PEGMA. After the desired reaction time, the PEGMA-grafted ePTFE membranes were transferred into a bottle with DI water. Unreacted macromonomers and homopolymers were extracted by soaking in methanol and DI water three times, and the solvent residue was removed. The surface modification with PEGMA macromonomer of all ePTFE membranes was performed under the same experimental processes.

2.3. Surface characterization

The chemical structure of ePTFE membrane modified with PEGMA was characterized with FT-IR spectrophotometer (Perkin-Elmer Spectrum One), using zinc selenide (ZnSe) as an internal reflection element. Each spectrum was captured from an average of 32 scans at a resolution of 4 cm^{-1}. The grafting degree of PEGMA on the ePTFE membrane is defined as the difference in weight between the modified ePTFE membrane and the pristine ePTFE membrane divided by the weight of the pristine ePTFE membrane. Weight measurements were performed using three independent membranes for each modified membrane, and the average value was reported. Water contact angles were measured at 25 °C with an angle-meter (Automatic Contact Angle Meter, Model CA-VP, Kyowa Interface Science Co., Ltd., Japan). A droplet of DI water was allowed to rest on the sample surface at five different sites. The average of the measured values from three independent membranes for each modified membrane was taken as its water contact angle. The surface morphology of the pristine and the surface-modified ePTFE membranes in liquid state was examined with bio-atomic force microscopy. All AFM images were acquired with a JPK Instruments AG multimode NanoWizard (Germany). The instrument is equipped with a NanoWizard scanner and operated in Liquid. For tapping-mode AFM, a commercial Si cantilever (TESP tip) of about 320 kHz esonant frequency from JPK was used.

2.4. Plasma protein adsorption on the membranes

The adsorption of human plasma solution of γ-globulin, fibrinogen, and albumin on the membranes was evaluated using the enzyme-linked immunosorbent assay according to the standard protocol described as follows. First, membranes of 1-cm^2 surface area were placed in individual wells of a 24-well tissue culture plate, and each well was equilibrated with 1000 μL of PBS for 60 min at 37 °C. Then, the membranes were soaked in 1000 μL of 100% PRP solution. After 180 min of incubation at 37 °C, the films were rinsed five times with 1000 μL of PBS and then incubated in bovine serum albumin (BSA, purchased from Aldrich) for 90 min at 37 °C to block the areas unoccupied by protein. The membranes were rinsed again five times with PBS, transferred to a new plate, and incubated in a 1000-μL PBS solution. The membranes were incubated with primary monoclonal antibody that reacted with the human plasma protein (i.e., HSA or Fg) for 90 min at 37 °C and then blocked with 10-mg/mL BSA in PBS solution for 24 h at 37 °C. The membranes were subsequently incubated with a secondary monoclonal antibody, horseradish peroxidase (HRP)-conjugated immunoglobulins, for 60 min at 37 °C. The primary antibody was not used and only the secondary antibody (goat $F(ab)_2$ anti-human immunoglobulin

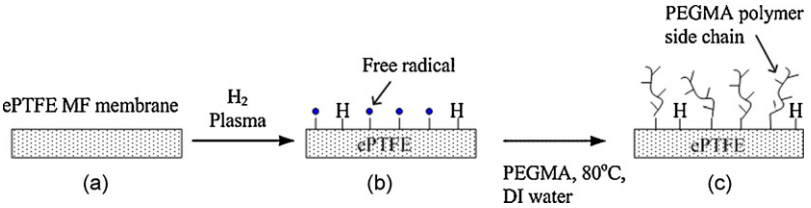

Fig. 1. Schematic illustration of the preparation process of the ePTFE-*g*-PEGMA membranes via surface copolymerization: (a) the ePTFE membrane cleaned via sonic in IPA and DI water at 25 °C, respectively, (b) the ePTFE membrane pretreated with H_2 plasma in vacuum at 25 °C, (c) the preactivated ePTFE membrane incubated in a DI water containing PEGMA macromonomer and thermally induced graft copolymerization at 80 °C.