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# Surface self-assembled zwitterionization of poly(vinylidene fluoride) microfiltration membranes via hydrophobic-driven coating for improved blood compatibility

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ABSTRACT

A facile surface self-assembled zwitterionization process is presented, aiming at improving the blood compatibility of poly(vinylidene fluoride) (PVDF) membranes. Zwitterionic diblock copolymers containing hydrophobic propylene oxide and hydrophilic sulfobetaine methacrylate blocks were prepared with well controlled molecular weights via atom-transfer radical polymerization (ATRP). Poly(propylene oxide)-block-poly(sulfobetaine methacrylate) (PPO-*b*-PSBMA) copolymers with varying zwitterionic PSBMA lengths were coated by self-assembling process onto the surface of PVDF microfiltration membranes in order to enhance their surface hydrophilicity and protein resistance. A systematic study regarding the effect of block lengths of PPO-*b*-PSBMA copolymers on the hemocompatibility of zwitterionic PVDF membranes in human blood solution was then performed. Protein adsorption from single-protein solutions and 100% blood plasma solutions was measured on the PVDF surfaces covered with PPO-*b*-PSBMA brushes. If the increase in PPO content in copolymer increased the efficiency of coating on the membrane, resistance to human fibrinogen adsorption was enhanced when increasing the PSBMA content. The control of the anchoring structures of zwitterionic copolymer layers highly regulates the adsorption of plasma proteins, the adhesion of platelets, and the coagulation of human plasma. PVDF membranes coated with PPO-*b*-PSBMA containing a high amount of zwitterionic SBMA units presented a high hydration capability, believed to allow improving significantly the hemocompatible character of PVDF membranes. This work suggests that the hemocompatible nature of self-assembled zwitterionic brushes gives them a great potential in the molecular design of antithrombogenic membranes for use in human blood applications.



## 1. Introduction

The design of hemocompatible membranes for blood contacting devices remains an actual challenge and the developments reported in this field over the past three decades aim at controlling the non-specific plasma protein adhesion and blood cellular attachment phenomena [1–5]. Indeed, as reminded by Sun and coworkers, protein adsorption on the surface of membranes happens rapidly after the first blood solution contact, and there are many severe consequences to this phenomenon [6]. In an effort to limit protein-adsorption and cell-attachment in blood-contact membranes, various factors need to be considered. Among those, the chemical nature of the membrane material influences the extent of low-energy interactions with biofoulants. It is generally acknowledged, regardless of the application of membranes, that hydrophilic surfaces tend to inhibit biofouling owing to proteins, bacteria and cells [7–12]. Because hydrophobic polymers such as polysulfone, polytetrafluoroethylene or polyvinylidene fluoride exhibit outstanding bulk properties, they are used in the design of anti-biofouling membranes. The creation of a hydrophilic interface enables the minimizing of the interactions with biofoulants [13,14]. Further, physical parameters have to be considered. Indeed, a smooth dense surface usually leads to lower water contact angles [15], offering very few anchorage sites so that adhesion of proteins and cells is limited. But in ultrafiltration or microfiltration, membranes are very porous so the roughness coefficient is logically not ideal to minimize the physical entrapment of biofoulants. In this respect, many efforts are being made aimed at developing and incorporating by blending, coating or surface chemical reaction polymer additives that can greatly

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enhance surface hydrophilicity of hydrophobic membranes [16–18]. In other words, the focus is laid on the membrane materials, rather than on the membrane morphology.

The common anti-fouling polymer additives for the resistance of protein adsorption and cell attachment onto membranes can be classified into two categories. The poly(ethylene glycol) (PEG)-type systems constitute one class [19–30]. However, PEG systems may face the problem of blood compatibility. To address this issue, a second category is composed of zwitterionic-type materials, which can be used in blood filtration membranes if the surface density and chain length of zwitterionic groups are controlled [31–34]. Chang and coworkers report that poly(sulfobetaine methacrylate) (polySBMA) has become the most widely studied zwitterionic polymer owing to its ease of synthetic preparation [35]. If the coverage of the surface is appropriately controlled and the charge neutrality of zwitterionic polymer brushes ensured, then the interactions with plasma proteins and blood cells are minimized, that is, hemocompatibility is ensured [36,37]. There is a wide variety of processes to achieve coverage of polymer membrane′s surface by zwitterionic additives. For instance, surface initiated atom transfer radical polymerization (ATRP) [11,36,38], surface-induced thermal graft polymerization [35], surface reaction [39], or UV-initiated polymerization [40] are among the possible methods. They ensure a good control of surface modification at a low scale. Yet, a more facile and cost saving method driven by hydrophobic interactions between the polymer membrane and additive molecules can also be efficient if copolymer brushes are supported by a strong hydrophobic anchorage group. We have reported the synthesis of diblock copolymers of poly(sulfobetaine methacrylate) [poly(SBMA)] and poly(propylene oxide) (PPO) [41]. The PPO blocks are susceptible to interact with any hydrophobic membrane material, so that physical interactions should be readily achieved via a simple coating process.

Following our previous work, the unique aspects of the present study are to test the feasibility of surface zwitterionization of poly (vinylidene fluoride) (PVDF) membranes by dip-coating using these novel copolymers, study the impact of the hydrophilic segment chain length on surface hydrophilicity, and move onto the investigation of their blood compatibility. Self-assembled anchoring of PPO-*b*-PSBMA onto PVDF microfiltration membranes was achieved. This is attributable to the establishment of hydrophobic–hydrophobic interactions between the PPO hydrophobic moieties groups and PVDF backbone. In this work, efficiency of hydrophobic driven coating is first assessed by weight measurements. Then, surface composition and morphology are characterized and discussed. Subsequently, the surface hydrophilicity of novel membranes is estimated. These results are then correlated to the ability of membranes to efficiently resist the adhesion of various proteins including bovine-serum albumin (BSA), lysozyme (LY), and fibrinogen (FN). To complete this work and conclude on the efficiency of PPO-*b*-SBMA coating to improve hemocompatibility of PVDF membranes in blood applications, platelets adhesion tests and plasma clotting time are finally evaluated.

## 2. Experimental

### 2.1. Materials

[2-(Methacryloyloxy)ethyl]dimethyl(3-sulfopropyl)-ammonium hydroxide (sulfobetaine methacrylate, SBMA) monomer, copper(I) bromide [Cu(I)Br], 2,2′-bipyridine (bpy), 2-bromoisobutyrylbromide, and triethylamine were purchased from Aldrich and used as received. Poly(propylene glycol) monobutyl ether (PPO–OH) with an average molecular weight of 1200 ($Dp_n = 20$), was also purchased from Aldrich. Tetrahydrofuran (THF; Aldrich, 99%) was distilled from sodium to keep it anhydrous. Fibrinogen (fraction I from human plasma) was purchased from Sigma Chemical Co. Human blood and plasma solution were obtained from the Taiwan Blood Services Foundation. Phosphate buffer saline (PBS) was purchased from Sigma-Aldrich. Bovine serum albumin (BSA), lysozyme (LY) and fibrinogen (FN) were purchased from Sigma Chemical Co. PVDF microporous membranes with an average pore size of 0.1 μm, a thickness of approximately 110 μm, and a diameter of 47 mm were purchased from the Millipore Co. (VVHP04700 and VVLP04700) and used as received. Deionized water used in the experiments was purified using a Millipore water purification system with a minimum resistivity of 18.0 MΩ cm.

### 2.2. Methods

#### 2.2.1. Preparation of PPO-b-PSBMA copolymers

Preparation of poly(propylene oxide)-block-poly(sulfobetaine methacrylate) (PPO-*b*-PSBMA) copolymers has been described elsewhere [41]. Briefly, polymerization is achieved via the ATRP method. PPO with a macroinitiator (PPO–Br) was synthesized by reacting PPO–OH with 2-bromoisobutyrylbromide (5:1, v:v) in THF, and the product was purified by extraction with brine three times. Subsequently, 5 g of SBMA were polymerized under nitrogen at 20 °C in 10 mL of methanol using the following [SBMA]/[PPO–Br]/[CuBr]/[bpy] molar ratio: 10:1:1:2, 20:1:1:2 and 40:1:1:2 in order to prepare three well-defined diblock copolymers. As the initial amount of SBMA was the same for the three reactions, that of PPO–Br – 4.89 g, 2.45 g or 1.22 g – permitted the controlling of the final molecular weight of the polySBMA block. After 24 h, the resultant reaction solution was passed through an aluminum oxide column, precipitated into ethanol and redissolved into water repeatedly to remove residue catalysts. A solvent was evaporated and the copolymer dried in a vacuum oven at room temperature to yield a white powder. The composition of block copolymers used in this work is reported in Table 1 and the chemical formula of PPO-*b*-PSBMA copolymer is displayed in Fig. 1. Aqueous GPC analysis indicated three PPO-*b*-PSBMA diblock copolymers with controlled polydispersities (i.e., Mw/Mn = 1.30–1.40). The molecular weights of the prepared PPO-*b*-PSBMA copolymers were determined by aqueous gel-permeation chromatography (GPC), using two Viscogel columns, a G4000 PWXL and a G6000 PWXL (the range of molecular weight was from 2 kDa to 8000 kDa) connected to a model Viscotec refractive-index detector at a flow rate of 1.0 mL/min and a column temperature of 23 °C. The eluent was an aqueous solution composed of 0.1 M $NaNO_3$ at pH 7.4. Poly (ethylene oxide) (PEO) standards from Polymer Standard Service, Inc. (Warwick, USA) were used for calibration.

#### 2.2.2. Surface self-assembled zwitterionization of PVDF microfiltration membranes

As-prepared zwitterionic copolymers were coated onto commercial microfiltration PVDF membranes, by a surface self-assembled zwitterionization process (Fig. 1). For the process of self-assembled coatings, clean PVDF membranes were soaked in an ethanol solution and then incubated for 120 min with various ethanol solutions containing PPO-*b*-PSBMA at a concentration in the range $0.1-3$ mg $mL^{-1}$. Membranes were rinsed three times with ethanol in 50% v/v water to remove weakly adsorbed copolymers. Finally, the membranes were rinsed three times in PBS and freeze dried at −45 °C under vacuum.

#### 2.2.3. Membrane characterization

Zwitterionization efficiency was assessed by evaluating the copolymer coating density (mmol/$cm^2$) of PVDF membranes. Surface compositions of virgin and coated membranes were