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Polysulfone based amphiphilic graft copolymers by click chemistry as bioinert membranes

Gorkem Yilmaz [a], Hojjat Toiserkani [a], Dilek Odaci Demirkol [b], Serhan Sakarya [c], Suna Timur [b,*], Lokman Torun [d], Yusuf Yagci [a,**]

[a] Department of Chemistry, Istanbul Technical University, 34469 Maslak, Istanbul, Turkey
[b] Ege University, Faculty of Science, Department of Biochemistry, Bornova, 35100, Izmir, Turkey
[c] Department of Infectious Diseases and Clinical Microbiology, Adnan Menderes University School of Medicine, 09100 Aydın, Turkey
[d] Chemistry Institute, TUBITAK Marmara Research Center, Gebze, Kocaeli, 41470, Turkey

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ABSTRACT

A series of well-defined amphiphilic graft copolymers with hydrophobic polysulfone (PSU) as backbones and hydrophilic poly(ethylene glycol) (PEG) as side chains were synthesized and characterized. For this purpose, PSUs were converted to azido-functionalized polymers by successive chloromethylation and azidation processes to give clickable PSUs. Then, the ω-hydroxyl function of the commercially available PEG-OH was converted into propargyl functionality by simple esterification process. Ultimately, the alkyne functionalized PEO was successfully grafted onto the PSUs by click chemistry. The final polymers and intermediates at various stages were characterized by ^{1}H NMR, FT-IR, and GPC techniques. The bioinert character of PEG grafted PSU was confirmed by static protein adsorption and prokaryotic and eukaryotic cell adhesion studies, and compared to that of unmodified PSU.

1. Introduction

Polysulfone (PSU) is one of the most popular thermoplastic polymeric materials widely used in the manufacture of synthetic polymer membranes because of its excellent properties such as outstanding oxidative, thermal and hydrolytic stabilities as well as good mechanical and film-forming properties due to the diarylsulfone group in the main chain backbone. PSU is a non-degradable, biocompatible polymer utilized as a membrane material in such applications as gas separation [1], pervaporation [2], hemodialysis [3], nano/ultra-filtration [4,5], drug delivery [6], bioartificial [7], ion-exchange membranes [8], cell culture [9] and so on. One of the major drawbacks of these synthetic membranes lies in their hydrophobic nature of PSU, which prevents their use in areas where prolonged body and membrane contact is required such as in the making of protective wears for chemical agents. Modification procedures allow finding a compromise between hydrophobicity and hydrophilicity, and localize the hydrophilic material specifically in the membrane pores, where they have a positive effect on flux and fouling reduction without compromising mechanical stability. In the past decades, many investigations have demonstrated that hydrophilic modification of hydrophobic membrane was an efficient strategy to overcome the drawback. For this reason, a variety of methods including bulk modification such as sulfonation [10] and carboxylation [11], surface modification such as preadsorption [12], plasma treatment [13] and grafting [14–17] and block polymerization [18–20] and polymer blending [21–23] have been explored to fabricate hydrophilic PSU membrane.

There are several examples for the preparation of polysulfone based graft copolymers with increased hydrophilicity. For example, Ulbricht and Belfort used radiation techniques to graft polymerize hydrophilic monomers such as 2-hydroxy-ethylmethacrylate, acrylic acid, and methacrylic acid onto PSU membrane surfaces. The resultant membranes showed increased flux and higher bovine serum albumin (BSA) retention than unmodified PSU membranes [24]. Higuchi and coworkers chemically grafted sulfonyl and hydroxyl end-terminated groups to PSU membrane surfaces and achieved reduced protein adsorption [25,26]. In another approach, Park and co-workers grafted PEG on PSUs by etherification process to yield porous network-like surface structure with enhanced porosity, wettability and resistance to protein adsorption compared with PSU membrane controls [27].

There are three general methods to prepare graft polymers: "grafting-onto" (the addition of previously prepared side chains to a backbone) [28–30], "grafting-from" (the polymerization of side chains from a macroinitiator backbone) [31–33], and "grafting-through" (the polymerization of macromonomers) [34–36]. The

* Corresponding author. Tel.: +90 232 3438624.
** Corresponding author. Tel.: +90 212 285 32 41.
E-mail addresses: suna.timur@ege.edu.tr (S. Timur), yusuf@itu.edu.tr (Y. Yagci).

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grafting onto approach can selectively be employed on backbones and side chains with well defined functionalities by successful coupling reactions.

The concept of "click chemistry" involving highly efficient coupling reactions was coined by Kolb et al. and Rostovtsev et al. [37,38] that is a well established synthetic tool in organic and macromolecular chemistry. The most popular click chemistry reaction is the copper-catalyzed Huisgen 1,3-dipolar cycloaddition between terminal alkyne and azide groups leading to 1,2,3-triazole under moderate reaction conditions. The high efficiency with a high functional group tolerance and solvent insensitivity makes this reaction particularly attractive for the synthesis of various macromolecular structures. In our laboratory, copper-catalyzed Huisgen 1,3-dipolar azide/alkyne, as well as Diels–Alder cycloaddition click reactions have been successfully used for functionalization of polymers [39–41], microsphere [42], clays [43] and silsesquioxanes [44] with thermal- [45–47], photo- [39], and electro active [44] groups. The azide–alkyne click reaction has also been used to modify surfaces of various solid supports including silica spheres [48,49], carbon nanotube electrodes [50,51] and glass surfaces [52–56], and crosslinked networks [57]. The 1,3-dipolar azide–nitrile cycloaddition has been used in the synthesis of well-defined polymeric tetrazoles [58]. Furthermore, step-growth click coupling of telechelic polymers was performed by using α-alkyne-ω-azido-terminated polystyrene [59]. Recently, we have demonstrated the possibility of using click chemistry strategy for successful functionalization of PSUs on the example of a model fluorescent molecule [60]. This way, the fluorescent properties were introduced to the PSUs by using pyrene propargyl as the click component.

Among the artificial materials for biomedical applications, PEG has been widely recognized as a water soluble, nontoxic, and bioinert polymer, mainly employed for its hydrophilic nature, which serves as an excellent coating material since it is compatible with biological systems and has been shown to reduce protein adsorption and cell adhesion on synthetic surfaces [61–65]. In the present work, we describe preparation of amphiphilic graft copolymers possessing hydrophobic PSU backbone and hydrophilic PEG side chains by click chemistry. The method described here is proposed as a practical surface modification strategy for the manufacture of semipermeable membranes used in medical devices. The biocompatibility of PEG grafted PSU is compared to that of unmodified PSU.

2. Experimental section

2.1. Materials

Polysulfone (PSU) (Udel P-1700) was used as received. N,N′-Dicyclohexylcarbodiimide (DCC, 99%, Aldrich), 4-dimethylaminopyridine (DMAP, 99%, Aldrich), 4-pentynoic acid (98%, Alfa Aesar), poly (ethylene glycol) monomethyl ether (Me-PEG, Mn = 550, Across), and dichloromethane (CH_2Cl_2, 99% Lab-Scan) were distilled over P_2O_5. *N*,*N*-Dimethylformamide (DMF, 99%, Aldrich), sodium azide (99%, Merck), copper (I) bromide (98%, Acros), 2,2′-dipyridyl (Acros Organics 99+%), paraformaldehyde (95%, Aldrich), chlorotrimethylsilane (≥99%, Aldrich), tin (IV) chloride (99%, Aldrich), methanol (99%, Reiden-de Haën), diethylether (98%, Carlo-Erba) and tetrahydrofuran (THF, 99% Lab-Scan) were used as received.

2.2. Synthesis of azidomethylated PSU (PSU-N_3)

PSU-N_3 was synthesized by using three-step procedure as described previously [60].

2.3. Preparation of ω-propargyl functionalized PEG (Pro-PEG)

Me-PEG (*M*n = 550) (0.55 g, 1 mmol) was dissolved in 25 mL of CH_2Cl_2. 4-Pentynoic acid (0.294 g, 3.00 mmol), DMAP (0.12 g, 1.0 mmol) and DCC (0.62 g, 3.0 mmol) in 5 mL of dichloromethane were added to the solution in that order. The reaction mixture was stirred overnight at room temperature. It was filtered and evaporated, and the remaining product was purified by column chromatography over silica gel eluting first with CH_2Cl_2/ethylacetate (1:1), and then with methanol/CH_2Cl_2 (1:10). Finally, the organic phase was evaporated to give Pro-PEG.

2.4. Preparation of PSU-g-PEG (PSU-g-PEG) via click chemistry

To a Schlenk tube equipped with a magnetic stirring bar, PSU-N_3 (0.20 g, 26,000 g/mol, 1.0 equiv), Pro-PEG (0.182 g, 550 g/mol, 3.3 equiv), ligand (2, 2′-dipyridyl, 0.33 mmol), catalyst (CuBr, 0.33 mmol) were dissolved in 10 mL of DMF. The tube was degassed by three freeze–pump–thaw cycles, left under vacuum, and placed in a thermostated oil bath. After the reaction, the mixture was diluted with THF and then passed through a column of neutral alumina to remove metal salt. The reaction mixture was concentrated and precipitated into methanol. The final product was washed with methanol and dried for 24 h in a vacuum oven at 25 °C. Yield = 0.212 g (83%).

2.5. Protein and cell adhesion studies

Protein and cell adhesion were tested on the membranes of the PSU and PSU-*g*-PEG polymers by using bovine serum albumin (BSA) and HuTu-80 human duodenum adenocarcinoma cell line as the model. For the membrane formation, polymers (10 mg) were dissolved in THF (1.0 mL) and degassing under vacuum. Then, 100 μL of each polymer solutions were cast into the 24-well tissue culture polystyrene (TCPS) plates using epoxy and allowed to dry at room temperature over night.

For the protein adsorption experiments, membranes were initially washed with phosphate-buffered saline solution (PBS, 0.01 M, pH 7.4) for 1 h, then incubated at room temperature in 0.01 M PBS containing 1.0 mg/mL BSA for 120 min, then washed with PBS and deionized water, respectively for 10 min. The amount of adsorbed protein on the membranes was estimated by following the initial and final concentrations of protein within the adsorption medium and washing solutions using Coomassie Brilliant Blue with crystalline BSA as standard [66].The experiments were performed in replicates of three. For each set of data present, standard statistical methods were used to determine the mean values and standard deviations.

HuTu-80 human duodenum adenocarcinoma cell line obtained from the American Type Culture Collection (Manassas, Va.) was grown in minimum essential medium (Eagle) with fetal bovine serum (10%) in the absence of antibiotics at 37 °C in 5% CO_2 and cultured to confluence. Confluent monolayer were spitted with Trypsin–EDTA treatment and suspended in medium at 10^5 cells/mL density. Cell suspensions were seeded in wells coated with PSU-*g*-PEG and PSU membranes and incubated at 37 °C in 5% CO_2 for 24 h. After incubation wells were rinsed with PBS twice and adherent cells examined with invert microscopy with 200× magnification.

Staphylococcus aureus 25923 strain was obtained from the American Type Culture Collection (Manassas, Va.) and cultured on blood agar (Difco Laboratories, Detroit, MI, US). The bacteria concentration of ~10^7 CFU mL^{-1} was monitored spectrophotometrically as optical density of 0.1 at A_{625} nm. The bacteria suspensions were prepared in Tryptic soy broth with a final concentration of 10^7 CFU mL^{-1} and 1 mL bacteria was cultured in wells coated with PSU and PSU-*g*-PEG membranes as well as uncoated wells as the negative control and incubated at 35 °C for 18 h. After incubation, wells were rinsed with PBS twice and adherent bacteria were detached with sonication and pipette agitation in 200 μL PBS. 50 μL of suspension were diluted appropriately (1/10, 1/100 and 1/1000) and plated on blood agar. Following 24 h incubation at 35 °C, the total number of bacteria for each dilution was counted.