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Surface modification of a polyethylene film for anticoagulant and antimicrobial catheter

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ABSTRACT

A functional anticoagulant and antibacterial coating for polyethylene (PE) films is described. The stepwise preparation of this nanocomposite surface coating involves O_2 plasma etching of PE film, carbodiimide coupling of cysteamine to the etched PE film, binding of Ag to sulfhydryl groups of cysteamine, and assembly of heparin capped AgNPs on the PE film. The nanocomposite film and its components were characterized by ^{1}H-nuclear magnetic resonance spectroscopy, attenuated total reflectance-Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy, and field emission-scanning electron microscopy. The resulting PE films demonstrate anticoagulant activity using a hemoglobin whole blood clotting assay, and anti-bacterial activity against *Bacillus cereus* 3551 (Gram-positive) and *Escherichia coli* BL21 (Gram-negative) bacteria. The hydrophilicity of the heparin coated PE was determined by contact angle measurements; and the stability of the nanocomposite film, with respect to Ag leaching, was assessed by atomic absorption spectroscopy.

1. Introduction

The presence of an indwelling central venous catheter (CVC) is the strongest independent predictive factor for thrombosis in the arm [1] and is considered the main risk factor for occurrence of upper extremity deep vein thrombosis (UEDVT) [2]. The morbidity and mortality ranges from 15 to 50% and are statistically equivalent to lower extremity deep vein thrombosis [3,4]. Post-thrombotic syndrome occurrence with DVT of the arm may be as high as one out of every three patients [5–7].

Radiographic studies show that up to 90% of cellular deposits form on the surface of the catheter, creating a fibrin sheath within the first 24 h after insertion [8]. Upper extremity thrombosis associated with peripherally inserted central catheters (PICCs) is becoming more common with the increased use of triple lumen catheters. Cancer patients with a CVC have greatly higher risk of developing UEDVT in the arm [9].

The relationship between thrombosis and infection has been established with significant colonization in areas of clot, and higher rates of catheter-related sepsis and catheter-related septicemia when thrombosis is present. In animal studies, fibrin sheath formation around central venous catheters significantly promoted colonization, catheter-related infection and persistent bacteremia [10–11].

According to the American College of Chest Physicians (ACCP Guidance, 2012), for most patients with UEDVT, catheter removal is not recommended if the device is functional and there is continued need for use. The damage associated with thrombosis is already done, but concerns over greater risk for infection remain. The rate of recurrence of upper extremity thrombosis if the catheter is removed and immediately placed into another site may be as high as 86% [12].

Catheters are used in many modern medical procedures [13], and a common use for catheters, such as a central venous catheter or Swan–Ganz catheter, involves their insertion into blood vessels [14]. The presence of such indwelling catheters can pose risks of blood clotting and infection [15–17]. Polymers used in making catheters are usually hydrophobic and often require lubrication to decrease damage on insertion into hydrophilic tissues [18]. In an effort to begin addressing these issues, our laboratory decided to investigate the use of stable hydrophilic nanocomposite coatings with anticoagulant and antibacterial properties.

Polyethylene (PE) based plastics are often used in the preparation of catheters [19]. Catheters are often made of the low-density PEs as these have more branching than high-density PEs and, thus, higher resilience. Because of these side branches, its molecules are less tightly packed and less crystalline. Low-density PE has a number of good performance characteristics, including transparency, flexibility, toughness, ease of processing, and an excellent ability for molding, making it suitable for use in catheters. PE, while having excellent mechanical properties, is

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quite hydrophobic [19], binds microbes [7,8,10,11], and has poor blood compatibility, resulting in rapid clot formation [9]. While the direct non-covalent modification of PE with anticoagulant, anti-bacterial layers is possible, covalent modification should offer a more stable coating. Unfortunately, PE does not contain reactive functional groups onto which anticoagulant or antimicrobial agents can be covalently attached. There are previous reports of introducing reactive functional groups onto the surface of PE films and catheters using O_2 plasma etching [20]. This modification introduces a surface layer of carboxyl groups onto PE that can allow for covalent attachment of a coating and increase the surface hydrophilicity without markedly changing the mechanical properties of the underlying PE.

The most commonly used anticoagulant is heparin, a polysaccharide-based drug that prevents blood from clotting by binding to the plasma protein antithrombin III (AT) and activating it against blood serine proteases, such as thrombin, that ultimately convert the soluble blood protein fibrinogen into an insoluble fibrin clot [21]. Heparin has been widely used to prepare coatings for catheters and other blood compatible devices used in medicine [21,22].

There are many approaches for the preparation of antimicrobial and antibacterial coatings [23]. The most widely used approaches rely on molecules that non-specifically and rapidly kill microbes. Silver nanoparticles (NPs) have recently commanded much attention as effective broad-spectrum antimicrobial agents [24–29]. Since there are some concerns about the use of free silver nanoparticles because of their toxicity [30], attention has turned to immobilized Ag NPs [31].

A single nanocomposite [32], showing a combination of anticoagulant and antibacterial properties, has been previously explored by our laboratory [33,34]. The current study examines the assembly of a covalently attached nanocomposite with hydrophilic coating on the surface of PE films, which exhibits both anticoagulant and antibacterial activities.

2. Experimental

2.1. Materials

Heparin sodium from porcine intestinal mucosa was purchased from Celsus Laboratories (Cincinnati, OH, USA). 2,6-Diaminopyridine (DAP), sodium cyanoborohydride, polyethylene (PE, average MW 35,000 determined by gel permeation chromatography (GPC) was selected for this study since it belongs to the class low-density PEs and is suitable for the experiments undertaken in the current study), cysteamine (aminothioethanol), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimidehydrochloride (EDC), Drabkin's reagent and other common reagents were purchased from Sigma Chemicals (St. Louis, MO, USA) and used as received. Silver nitrate ($AgNO_3$) was ordered from Amrensco (Solon, OH, USA). *N*-hydroxysuccinimide (NHS) was from Thermo Scientific (Rockford, IL, USA). Dialysis membrane, 1000 molecular weight cutoff (MWCO), was from Spectrum Laboratories, Inc. (Houston, TX, USA). Glass slides were from Fisher Scientific (Pittsburg, PA, USA).

2.2. Surface modification of PE film (see Scheme 1)

2.2.1. Synthesis of 2, 6-diaminopyridinyl heparin (DAPHP)

The method of synthesizing 2, 6-diaminopyridinyl heparin (DAPHP) is described elsewhere in detail [35]. Briefly, heparin (500 mg, 41.5 μM) was dissolved in 15 mL formamide by heating at 50 °C. 2, 6-Diaminopyridine (500 mg, 4600 μM) was added and the reaction was maintained at 50 °C for 6 h. Aqueous sodium cyanoborohydride (47.5 mg, 750 μM) was added and the mixture was incubated at 50 °C for an additional 24 h. The reaction mixture was diluted with 50 mL of water and dialyzed against 2 L of water for 48 h using a 1000 MWCO dialysis membrane. The retentate was recovered, lyophilized, and purified by methanol precipitation three times. After the final precipitation, the precipitate was dissolved in water, dialyzed (1000 MWCO) against 2 L of water for 48 h twice, and lyophilized.

2.2.2. Surface modification of PE film

The PE film was prepared by cast melting. The polyethylene particles were put on a silicon wafer and heated at 160 °C. When melted, the PE was covered with a pre-cleaned microscope slide. The hot PE "sandwich" was immediately taken off the hot plate. After the PE cooled down sufficiently to solidify, the glass slide and silicon wafer were scraped with a razor blade to obtain a PE film having a thickness of ~0.5 mm.

The PE films, thus prepared, were treated with oxygen plasma (oxygen pressure 0.5–1.2 Torr) at a variety of power settings (30 watts, 50 watts, 100 watts) and for different lengths of time (30 s or 60 s) to afford surface carboxyl functionality using a TEGAL 411 plasma barrel stripper (Tegal Corp., Petaluma, California, USA). The oxygen plasma-activated, carboxyl-functionalized, PE film was then activated by immersing in an aqueous solution of 16 mmol/mL EDC and 16 mmol/mL NHS, and gently shaken for 1 h. When the reaction was completed, the films were then transferred into aqueous solution of cysteamine (0.05 M) and shaken for another 3 h. PE films modified with thiol functional groups on their surface were obtained and subsequently transferred into $AgNO_3$ solution (140 mM) for 1 h incubation to bind Ag^+ ions to the thiol groups.

PE-Ag nanoparticles (NPs) were synthesized by reduction with DAPHP [33]. The PE films were placed standing upright in a flask. DAPHP aqueous solution (reducing agent) was then added into the system (0.5 mM solution) and the mixture was heated at 95 °C for 4 h. The PE films changed color to brown indicating the growth of AgNPs. Afterwards, clear DAPHP-AgNPs coated PE films were obtained after three-time wash with water and air-dried.

2.3. Characterization

The ^{1}H NMR spectra were obtained on a Bruker 600 MHz spectrometer (Bruker, Switzerland) with Topsin 2.1 software. All measurements were performed at 298 K, using the pulse accumulation of 64 scans and LB parameter of 0.30 Hz. Deuterium oxide (D_2O) was used as the solvent for DAPHP. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were collected with a PerkinElmer Spectrum One Spectrometer (PerkinElmer, Inc. Waltham, Massachusetts, USA) using the Diffuse Reflectance sampling accessory with a Zinc Selenide internal reflection element (IRE). These spectra were collected using rapid-scan software spectrum v5.3.0 with 8 scans and a resolution of 4 cm^{-1}. X-ray photoelectron spectroscopy (XPS) measurements were carried out in a PHI 5400 instrument with a 102 W Al KR probe beam. The spectrometer was configured to operate at high resolution with a pass energy of 20 eV. Samples were imaged using a field emission scanning electron microscope (FE-SEM) of a Zeiss SUPRA-55 instrument (Oberkochen, Germany). All samples were sputter-coated with 1 nm of Pt (Denton Vacuum Desk IV, Moorestown, NJ) prior to imaging. Images were obtained at a working distance of 2.8–4.7 mm using an acceleration voltage of 2 kV or 10 kV. Energy dispersive X-ray (EDX) microanalysis was performed on Oxford INCA EDS System 250 equipped in a FE-SEM Zeiss SUPRA-55. The electron beam was operated at 15 kV.

2.3.1. Anticoagulant activity of the film surface

Anticoagulant activity was determined by using a hemoglobin (Hb) assay to measure the hemoglobin content in the red fibrin clot formed on the PE film after applying smear of human whole blood. The negative (−) control film (unmodified PE) showed the highest Hb concentration and the positive (+) control film (PE with heparin) showed Hb concentrations approaching zero. Using 96-well plate plastic sealers, PE films (unmodified, treated with O_2 plasma or coated with DAPHP-AgNPs) were cut into dimensions of 2 cm × 2 cm and were placed into 6-well, sterile, clear, tissue culture plates. For (+) control samples, 50 μL of known concentrations of heparin (0.6 mg/mL, 0.9 mg/mL, 1.5 mg/mL)