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Complement activation by sulfonated poly(ethylene glycol)-acrylate copolymers through alternative pathway

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Abstract

Previously, novel poly(ethylene glycol) (PEG) and sulfonated PEG acrylate (PEG-SO_3A/OA) copolymers were prepared as coating and/or blending materials for biomedical applications. Surfaces modified with copolymers exhibited increased anti-coagulation properties and decreased plasma adsorption level due to increased hydrophilic properties and reorientation characteristics of PEG/PEG-SO_3A chains in water phase. As continuation study, anti-complement effects of PEG-SO_3/OA copolymers were investigated in vitro, and compared with those of low-density polyethylene (LDPE) and PEG/OA. C3 activation by PEG-SO_3/OA samples was lower than that by PEG/OA samples, which was attributed to decreased surface nucleophile level of samples. PEG-SO_3/OA samples increased inhibition of Bb production, resulting in decreased C5 activation. Owing to reduced activations of C3 and C5, PEG-SO_3/OA samples markedly decreased SC5b-9 levels in plasma.

Keywords: Biomaterials; Poly(ethylene glycol); Acrylate; Copolymerization; Sulfonation complement activation; Alternative pathway

1. Introduction

The use of blood-contacting biomaterials has been challenged by diverse and complex reactions of the blood components to the biomaterials [1]. Plasma protein adsorption [2–5], thrombogenesis [6–9], and complement activation [10–12] are indications of a myriad of reactions of the blood against foreign materials. In this context, extensive investigations have been undertaken on the modification of biomaterial surfaces with the prospect of attaining hemocompatibility [13,14], including the grafting of water-soluble polymer chains, such as PEG, onto the surface of biomaterials [2,4,11,13]. Grafting of PEG provides a hydrophilic environment with low interfacial free energy between the interface of the blood and materials, resulting in reduced protein adsorption, and platelet adhesion and activation [15]. In addition, the introduction of negatively charged pendent group, if applicable, further expels the blood components by electrical repulsion [16].

In a previous study, the concept of negatively charged PEG was extended to the preparation of a copolymer system, through which a series of copolymers containing a hydrophilic part of PEG/PEG-SO_3 acrylate and a hydrophobic part of octadecylacrylate (OA) were prepared as coatings or processing additives for medical devices and implants [17]. The surfaces of these copolymers demonstrated remarkable antithrombogenic properties with significantly reduced plasma protein adsorption levels.

Direct thrombogenic response of plasma, or platelet in short, is the most significant aspect of the blood compatibility of a material. However, for longer duration implants, other components of the host response to foreign objects may also have significant effects on the biocompatibility of the implant [18].

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The complement system, which is essential to the recognition of pathogenic agents, can be activated non-specifically through an alternative pathway in most foreign surfaces; activation of the complement system has been associated with increased thrombogenesis and leukocyte adhesion to the activating surface [18]. It is thus important to examine the complement-activating potential of copolymers, because PEO-grafting has been one of the major approaches used to achieve surface biocompatibility. Therefore, in the present study, complement activations of the fluid phase components, C3a, Bb, iC3b, C5a, and C5b-9, and the bound phase components, C3c, C3d, and C5b-9, were assessed by enzyme-linked immunosorbent assay (ELISA) and immunoblot, respectively, to investigate the anti-complement effects of PEG-SO_3/OA copolymers.

2. Experimental

2.1. Preparation of monomers

PEG mono-acrylates (PEGA, Monomer-Polymer and Dajac Lab., Feasterville, PA, USA) were dissolved in chloroform, precipitated in diethyl ether, and vacuum-dried at room temperature. OA (Aldrich Chemical Co.) and α,α′-azobisisobutytyronitile (AIBN) were purified by vacuum-distillation, and recrystallization using methanol, respectively. PEG-SO_3A monomers were obtained through sulfonation of PEGA monomers (Mws of PEGs: 1, 2, and 4 K) using Na metal and propane sulfone, as described elsewhere [17]. PEG-SO_3A monomers were dissolved in distilled water, precipitated in excess diethyl ether, and vacuum-dried at room temperature.

2.2. Synthesis of poly(PEG-SO_3A/OA)

Copolymers were synthesized through free radical polymerization of PEGA/OA and PEG-SO_3A/OA monomers in toluene using AIBN as an initiator [15]. Briefly, the polymerization solution was prepared by dissolving 20 wt.% monomers and 0.15 wt.% AIBN into toluene in a three-necked flask equipped with a condenser, a thermo-controller, and a magnetic stirrer. Nitrogen gas was purged for 30 min to deoxygenate the reaction solution, and polymerization was carried out by decomposition of AIBN at 70 °C for 48 h. The reaction volume was then reduced to ∼60% by solvent evaporation under reduced pressure, precipitated in an *n*-hexane or diethyl ether, and vacuum-dried at room temperature for 2 days. The structures and composition of copolymers are shown in Fig. 1.

2.3. Preparation of copolymer-coated tubings for fluid phase assay

Inner surface of the commercialized low-density polyethylene tubing (3.2 mm I.D., 6.4 mm O.D., Nalgene 489) was coated by pumping the copolymer solution (1% wt./vol. in dimethylacetamide) through the tubing using a peristaltic pump (80EL004, Millipore Co., Bedford, MA, USA) for 30 s, followed by 3 h drying under peristaltically pumped air and 24 h vacuum-drying at 40 °C. The procedure was repeated to ensure uniform coating. The prepared tubings were peristaltically washed and rinsed with phosphate buffered saline (PBS, pH 7.4) for 15 min to remove additives as well as to provide the hydration state of surface before fluid phase assay.

2.4. Preparation of copolymer-coated films for bound phase assay

Low-density polyethylene film (LDPE, Abiomed Inc., Danvers, MA, USA) was used as a negative control. Copolymers were cast from the solution (1% wt./vol. in dimethylacetamide) on both sides of the LDPE film surfaces and vacuum-dried at 40 °C.

2.5. Immunoassays of fluid phase complement components

Fresh whole human blood was collected from healthy donors using a vacuum tube technique for Routine Venipuncture, allowed to coagulate at room temperature for 30 min and centrifuged at 4 °C for 10 min [19]. The serum fractions were pooled into the polyethylene intravenous fluid bags (Choongwae

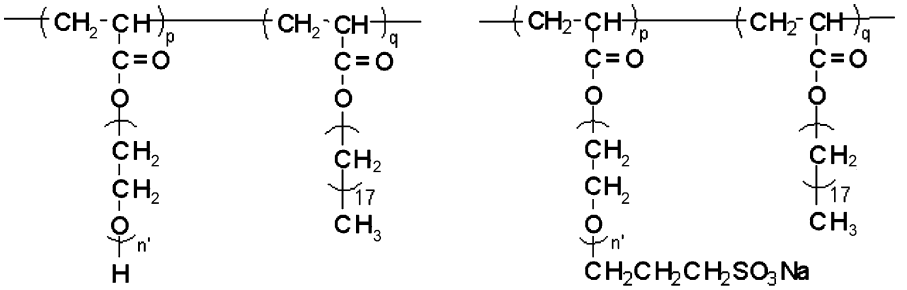

Fig. 1. Structure of the synthesized copolymer: *The composition of copolymers was estimated by ^{1}H-NMR as depicted in a previous study [17].