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In vitro anticoagulant activity of polyanionic graft chains modified poly(vinyl alcohol) particles

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ABSTRACT

Poly(acrylic acid), poly(sodium styrenesulfonate), and poly(acrylic acid-*co*-sodium styrenesulfonate) chains were immobilized onto poly(vinyl alcohol) (PVA) particles via a facile γ-ray simultaneous irradiation induced graft polymerization technique, which were confirmed by the attenuated total reflection Fourier transform infrared spectroscopy and the high swelling ratios of modified PVA particles. The effects of absorbed dose, dose rate, Cu^{2+} concentration and monomer concentration on the degree of grafting (DG) of PVA particles were investigated to find out a feasible process for preparing polyanionic chains graft-modified PVA particles. The clotting time results illustrated that both PVA-*g*-PAA and PVA-*g*-PSSS particles presented excellent anticoagulant activity, and the activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) were effectively prolonged with the increase of DG_{AA} and DG_{SSS}, respectively. Furthermore, the anticoagulant activity of PVA-*g*-PSSS samples was more efficient than that of PVA-*g*-PAA samples. However, the anticoagulant effect of PVA-*g*-P(AA-*co*-SSS) samples was different from that of PVA-*g*-PAA and PVA-*g*-PSSS samples, and was similar to that of heparin, mainly elongating the APTT and TT. This might be due to both of them containing the same negative-charged groups. Additionally, the grafted PVA particles were all non-hemolytic, showing good blood compatibility.

1. Introduction

Heparin, a kind of glycosaminoglycans with higher negative charged density, has been commonly used as clinically injectable anticoagulant to suppress blood coagulation and thrombin formation (Capila and Linhardt, 2002). The anticoagulant mechanism of heparin is deemed to that the heparin molecule containing carboxyl and sulfonate groups can induce the combination of antithrombin III (AT III) and thrombin (TB) to its specific sites, and the bound AT III could react with the bound TB to prevent blood coagulation (Hirsh et al., 1998). Currently, on account of the artificial biomedical materials widely employed for implants, artificial organs and tissue regeneration, surface heparinization of biomedical materials has been extensively carried out through various methods, such as blending, surface coating, as well as layer-by-layer assembly, in order to improve their anticoagulant activity to overcome the potential threat, blood coagulation and thrombin formation induced by foreign materials contacting blood (Liu et al., 2014).

Nevertheless, concerning with (1) the animal origin of heparin easy to be contaminated, (2) the heparin dosage difficult to control during clinical treatment process, and (3) a complex formation between heparin and platelet factor 4 inducing severe autoimmune response, it is imperative to develop alternative and inexpensive anticoagulants from other sources with more expected bioactivity and low side effects (Oh et al., 2013; Wang et al., 2009). The structurally well defined and non-mammalian source of heparin derivative is one of the best options, which have attracted considerable interest. A number of synthesized anticoagulants with sulfonate and carboxyl groups exhibit good anticoagulant activity (Cheng et al., 2014). However, as compared the anticoagulant effect of carboxyl group with that of sulfonate group, Jung et al. (2009) illustrated that the disulfonated poly(ethylene oxide) exhibited the heparin-like anticoagulant activity, which was in accordance with the poly(sodium styrenesulfonate) (PSSS) chains (Lin et al., 2009) and poly(acrylic acid-*co*-sodium styrenesulfonate) (P(AA-*co*-SSS)) chains (Xiang et al., 2015), while the poly(ethylene oxide)-dicarboxylic acid did not show any anticoagulation activity. Nonetheless, Nie et al. (2014) confirmed that the both carboxyl and sulfonate groups modified silicon wafer displayed anticoagulant effect via prolonging the activated partial thromboplastin time (APTT). Furthermore, for the anticoagulant effect of carboxyl group from

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poly(acrylic acid) (PAA) chains, Monien and Desai (2005) synthesized linear PAA chains exhibiting good anticoagulant acitivity, which could bind to antithrombin and accelerate the inhibition of factor Xa and TB; on the contrary, Lin et al. (2005) showed the PAA chains grafted thermoplastic polyurethane membrane devoid of anticoagulant effect. This consequence might be attributable to that, compared with that of untethered PAA chains, the anticoagulant activity of end-tethered PAA chains is weakened owing to the conformational change. This is similar to that covalent immobilization of heparin confines the native conformation of heparin and thus weakens its bioactivity (Yang et al., 2012). Therefore, an comparative research should be conducted in order to elucidate the difference of anticoagulant activity among the polyanionic chains, including PAA, PSSS, and P(AA-*co*-SSS) chains, which, to the best of our knowledge, has not been systematically performed until now. This work would be of great help to prepare more efficient and safety anticoagulants in future.

Polyanionic chains are usually grafted onto biomedical materials for improvement of anticoagulant activity, since this approach enhances the stability and persistence of polymer chains (Cheng et al., 2014). Hence, the graft polymerization method is adopted in this research in order to realistically and truthfully investigate the anticoagulant effect of the three kinds of polyanionic chains. SSS is difficult to graft polymerized onto the majority of polymeric materials because of (1) the highly ionized sulfonated groups surrounded by a large hydration spheres resulting in the incompatibility between the hydrophilic sulfonated group and hydrophobic surface of polymeric materials (Nasef et al., 2011) and (2) high negative charge over a broad pH range leading to a strong repulsive electrostatic force between the growing PSSS chains and SSS monomers. Nonetheless, SSS can be graft polymerized onto the polar poly(vinyl alcohol) (PVA) via γ-ray simultaneous irradiation inducement or cerium salt initiation (Gao et al., 2013), without the aid of other monomers. Besides, PVA has been broadly used in the field of biomedical materials through various methods, resulted from its nontoxicity and biocompatibility (Baker et al., 2012).

As a result, in this work, three types of polyanionic chains, including PAA, PSSS, and P(AA-*co*-SSS) chains, are grafted onto PVA particles via γ-ray mutual irradiation induced graft polymerization of AA, SSS and AA/SSS, respectively. The γ-ray direct irradiation technique is applied as a result of that (1) the degradation of PVA in aqueous solution induced by γ-ray direct irradiation can be effectively inhibit under the protection of H_2O and monomers (Takács et al., 2007), and (2) the graft polymerization reaction can be carried out at room temperature, which does not lead to the dissolution of PVA particles in aqueous solution. APTT, prothrombin time (PT) and thrombin time (TT) of human poor platelet plasma (PPP) contacted by the grafted PVA particles are utilized to evaluate the anticoagulant activity in order to reveal the difference of anticoagulant effect among the three kinds of polyanionic graft chains. Besides, the hemolytic ratio (HR) is used to estimate the compatibility between the synthesized PVA particles and erythrocyte in order to preliminarily assess their blood compatibility.

2. Materials and methods

2.1. Materials

PVA particles with irregular shape (diameter: 2–8 mm; degree of polymerization: 1700 ± 50, degree of alcoholysis≥99%), acrylic acid (AA, AR) and copper sulfate pentahydrate ($CuSO_4·5H_2O$, AR) were purchased from Sinopharm Chemical Reagent Co., Ltd., China. SSS (CP) was bought from Zibo Hongqi Chemical Plant, Shandong, China. Normal saline (Sichuan Kelun Pharmaceutical Co., Ltd., China), human red blood cell (washed for three times) and PPP were kindly offered by Chengdu Blood Center, Sichuan, China. All other reagents were of analytical grade, and used without further refinement.

2.2. γ-ray simultaneous irradiation induced graft polymerization

Firstly, PVA granules were immersed into AA, SSS, and AA/SSS deionized aqueous solutions in different glass tubes, respectively. Each tube contains 80 g of PVA particles and 650 mL of monomer aqueous solution. $CuSO_4·5H_2O$ was dissolved in AA and AA/SSS aqueous solutions as the homopolymerization inhibitor. The tubes were sealed and irradiated by ^{60}Co γ-ray at room temperature after bubbling with nitrogen for 30 min to remove oxygen from the aqueous solutions. After that, the grafted PVA granules were taken out and washed with deionized water to remove residual monomers and homopolymer. Finally, the modified PVA granules were dried at 50 °C under vacuum until constant weights were obtained. In the control, the raw PVA particles in deionized water were irradiated by γ-ray, and no evidence showed a decrease in the weight of the irradiated PVA particles with the enhancement of absorbed dose from 2.6 to 30.4 kGy (dose rate: 0.44 kGy/h). Consequently, the degree of grafting of AA (DG_{AA}) of PVA-*g*-PAA particles and the degree of grafting of SSS (DG_{SSS}) of PVA-*g*-PSSS particles were calculated by Eq. (1) and Eq. (2), respectively:

$$DG_{AA}(\%) = W_1 - W_0/W_0 \times 100 \quad (1)$$

$$DG_{SSS}(\%) = W_2 - W_0/W_0 \times 100 \quad (2)$$

where W_0, W_1 and W_2 are the weights of the original PVA, PVA-*g*-PAA and PVA-*g*-PSSS particles, respectively.

The DG_{SSS} of PVA-*g*-P(AA-*co*-SSS) particles was measured by acid-base titration method according to Eq. (3). The dried PVA-*g*-P(AA-*co*-SSS) particles were firstly immersed into hydrochloric acid (HCl, 1 mol/L) deionized aqueous solution for 24 h to convert the sodium sulfonate group ($-SO_3Na$) into the sulfonic acid group ($-SO_3H$). After that, the particles were washed with deionized water to remove the adsorbed HCl solution, and put into sodium chloride (NaCl, 5 wt%) deionized aqueous solution for 24 h with stirring after dried in a vacuum oven at 50 °C. The H^+ concentration in the NaCl solution was titrated with NaOH solution (0.01 mol/L, standardized by potassium acid phthalate).

$$DG_{SSS}(\%) = C_{NaOH} \times V_{NaOH} \times 206/W_0 \times 100 \quad (3)$$

where C_{NaOH} is the concentration (0.01 mol/L) of NaOH solution, V_{NaOH} is the volume of NaOH solution titrating the NaCl solution, and W_0 is the weight of pristine PVA particles.

The DG_{AA} of PVA-*g*-P(AA-*co*-SSS) particles was calculated by Eq. (4):

$$DG_{AA} = DG - DG_{SSS} \quad (4)$$

where DG is the total DG of PVA-*g*-P(AA-*co*-SSS) particles, and DG_{SSS} is the same as in Eq. (3).

2.3. Characterization

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of the pristine and modified PVA particles, ranging from 4000 to 600 cm^{-1}, were acquired from a Bruker Tensor 27 FT-IR spectrometer by averaging 32 scans at a resolution of 4 cm^{-1}.

The swelling ratio (SR) of PVA particles was assessed according to the method reported in the literature (Basfar and Lotfy, 2015). The dried PVA particles were immersed into deionized water at room temperature. The tested specimens were weighed at different time intervals after treated with filter paper under appropriate conditions of pressure. In addition, the photographs of PVA samples in deionized water at various intervals of time were recorded. Each experiment was repeated three times to obtain an average value. The SR was determined by Eq. (5),

$$SR(\%) = W_s/W_i \times 100 \quad (5)$$

where W_s and W_i are the weights of the swollen and the initial PVA particles, respectively.