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Construction of anticoagulant poly (lactic acid) films via surface covalent graft of heparin-carrying microcapsules

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ABSTRACT

Heparin has been widely investigated in medicine and biomedical applications as an anticoagulant. This paper described a covalent modification of poly (lactic acid) (PLA) films with heparin-carrying microcapsules. The heparin-carrying microcapsules were achieved by layer-by-layer (LbL) self-assembly technology by which poly (allylamine hydrochloride) (PAH) and heparin were alternately coated on the Ca^{2+}-cross-linked alginate hydrogel. The microcapsules were characterized by scanning electron microscopy (SEM), zeta potential analysis and Fourier transform infrared spectroscopy (FTIR), and then grafted to PLA films by –CONH– linkage between surface PLA molecules with residual primary amino groups on the outer PAH layer of microcapsules, in the presence of catalysts of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS). SEM and FTIR revealed the formation of –CONH– linkage. In vitro antithrombogenicity by the method of the activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) tests showed that surface-modified PLA films had superior coagulation properties to original PLA films. It is suggested that the present method would provide a potential effective tool for biomaterial modification.

1. Introduction

Heparin is a biologically relevant, highly anionic glycosaminoglycan. It has been widely investigated in medicine and biomedical applications, including its common clinical use as an anticoagulant [1]. Heparin is able to reversibly bind to many bioactive proteins, such as antithrombin III, heparin interacting protein, and so on [2]. The ability of heparin to associate with a wide variety of proteins makes it an ideal candidate as a non-covalent cross-linker and drug delivery agent in hydrogels with controlled release profile [3].

Anticoagulant biomaterials with non-covalent cross-linked heparin usually keep heparin working in short time, which is not suitable for use in a flow environment with mechanical shears, especially in blood. The direct covalent graft between heparin and material can provide relatively long-lasting binding of heparin molecules, however, it may also utilize the active sites on heparin molecules and result in loss of its efficiency [4,5]. For example, Alferiev et al. have reported that surface-covalently bound-heparin retains only 11.7% of the activity of free heparin [6].

The layer-by-layer (LbL) self-assembly technology, because it can be accomplished under gentle conditions, such as room temperature and neuter aqueous solution, has been regarded as a great support to solve many problems in the fields of drug delivery, biosensors, and microreactors, as well as bioseparations [7,8]. In particular, various microcapsules applied in drug delivery have been manufactured through the LbL self-assembly technology [9–11]. On the other hand, alginate has been reported as suitable for many biological components, and it can form hydrogel microspheres via ionically cross-linking in the presence of multivalent cations [12,13]. This kind of hydrogel has porosity allowing high diffusion rates of macromolecules [14]. Since alginate contains carboxylic acid groups on polyguluronate units, alginate microspheres (AMs) exhibit negative surface charges, and they have been used as negatively charged templates for polyelectrolyte LbL assembly [15,16].

The aim of the present study is to construct a new kind of anticoagulant biomaterials by covalently grafting heparin-carrying microcapsules onto the surface of a biomaterial, using a processing route illustrated in Fig. 1. In this processing route, AMs were coated with {poly (allylamine hydrochloride) (PAH)/Heparin/PAH} layers by the LbL self-assembly technique to form heparin-carrying microcapsules, which were denoted PAH/Hep/PAH@AMs. Then the microcapsules were grafted onto poly (L-lactic acid) (PLA) films, by reaction of carboxylic acid groups of PLA on the surface of films with residual primary amino groups on the outer PAH layer

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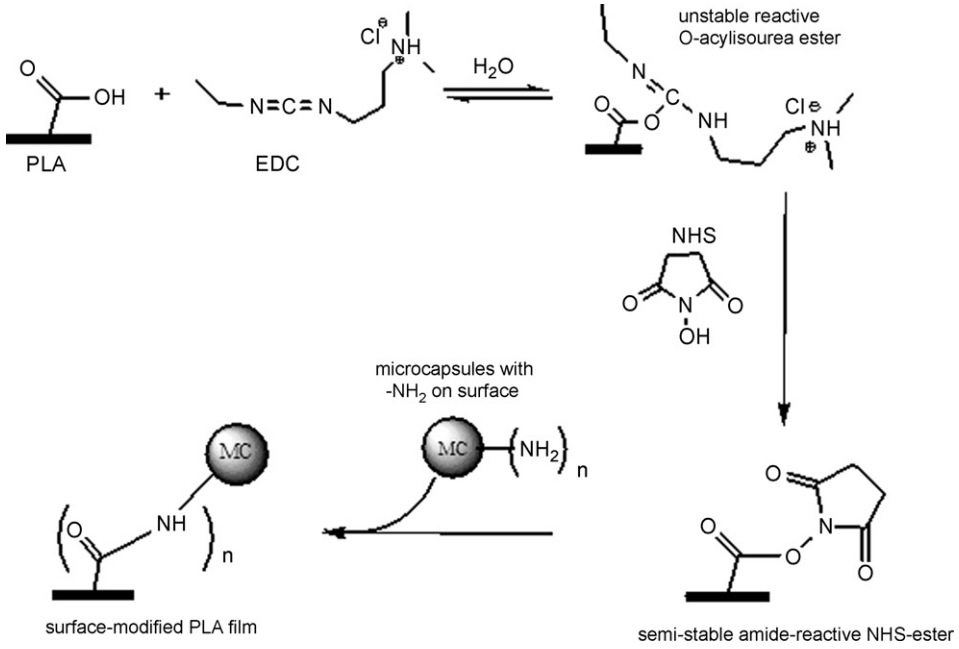

Fig. 1. Reaction scheme for surface modification of PLA films by covalent graft of heparin-carrying microcapsules.

of microcapsules using catalysts of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS). To determine whether microcapsules immobilization improved the blood compatibility of the modified films, in vitro antithrombogenicity was determined by the method of the activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) tests.

2. Materials and methods

2.1. Materials

Sodium alginate (low viscosity, 250 cps, and Mw 20–80 kDa), calcium chloride, sorbitan trioleate (SPAN 85), and polyoxyethylene sorbitan trioleate (TWEEN 85) were all purchased from Alfa-Aesar. 2,2,4-Trimethylpentane (isooctane) was purchased from Guangdong Chemicals Inc. PAH (Mw 60–70 kDa) was obtained from Sigma–Aldrich. EDC and NHS were from TCI AMERICA Tokyo Kasei Kogyo Co., Ltd. 1, 6-diaminohexane and heparin (Na salt, 150 USP units/mg) were purchased from Fluka and Beijing Aoboxing biotechnology Co., respectively. PLA (Mw 250–300 kDa) was a generous gift of Institute of Physical and Chemical Technology of Chinese Academy of Sciences. Kits for in vitro coagulation time tests (PT, APTT and TT) were purchased from Huashan Hospital of Shanghai Medical University. Human blood plasma was obtained from China Red Cross Association.

2.2. Methods

2.2.1. Fabrication of AMs and microcapsules PAH/Heparin/PAH@AMs

AMs were prepared by an emulsification technique described by Zhu et al. [16–18]. Briefly, 100 g 1.5 wt% sodium alginate aqueous solutions was dispersed in 75 g isooctane containing 1.7 g of SPAN 85 using an ultrasonicator (KUNSHAN Technology Co., Ltd.) at 100 W for 5 min. A solution containing 0.9 g of TWEEN 85 in 5 g of isooctane was then added to the emulsion and ultrasonicated at the same power for another 5 min to obtain stable water/oil emulsion droplets. Then 20 mL of aqueous solution containing 10 wt% of calcium chloride was added dropwise and stirred for 20 min. The resulted AMs were washed three times with deionized (DI) water. The AMs can be freeze-dried, and stored at room temperature.

For microcapsules preparation, PAH/Heparin was deposited by turns on AMs via LbL self-assembly [16]. Briefly, the sodium heparin and PAH solutions were prepared at a concentration of 2 mg/mL in deionized water containing 0.1 M $CaCl_2$ [16,19]. Then 1 mL of a polyelectrolyte solution was added to a microcentrifuge tube containing 100 μL of microsphere suspension containing $\sim 10^{12}$ microspheres, and incubated at room temperature for 20 min. After centrifugation, the pellets were harvested and washed with DI water for three times. The process was repeated to deposit oppositely charged polyelectrolyte, producing microcapsules PAH/Hep/PAH@AM.

2.2.2. Immobilization of microcapsules PAH/Heparin/PAH@AMs onto surface of PLA films

The PLA films were made by a hot-pressing process with a predetermined thickness of about 100 μm via a stainless spacer at 180 °C and a pressure of 25 MPa [20]. PLA films were rinsed with 0.05% NaOH to free more –COOH groups, and washed with water [21]. The –COOH groups on the surface of PLA films were activated by an EDC/NHS solution consisting of 5 mg/mL of EDC and 3 mg/mL of NHS in pH 5.0 sodium acetic buffer for 30 min at ambient temperature to create the stable and amine-reactive NHS-esters [22]. The activated PLA films were then rinsed with sodium acetic buffer (pH 5.0), and incubated for 4 h with 5 mg/mL alginate microsphere-templated microcapsules carrying heparin, which were pre-treated by 0.002 M triethylamine for 10 min to convert NH_4^+ to $-NH_2$ [19,22]. To remove non-covalent microcapsules and excess reaction reagents such as catalysts, the films were soaked in aqueous 10% SDS solution for 5 min and rinsed eight times by DI water successively. The films were subsequently air-dried for storage. Three kinds of controls were set up in the experiments, including PLA films not activated by catalysts, microcapsules not activated by triethylamine, and neither PLA films nor microcapsules activated. The experiment was repeated three times.

The hydrolytic experiment was performed in 0.01 M phosphate buffered saline (PBS, pH 7.4) containing 0.01% NaN_3. The PLA films with or without grafted microcapsules were immerged into 5 mL centrifuge tubes, and kept at 37 °C with gently shaking at 135 rpm for a week [23,24]. The films were characterized by attenuated total