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Hemostatic porous sponges of cross-linked hyaluronic acid/cationized dextran by one self-foaming process

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ABSTRACT

Effective hemostatic materials are very important for treating trauma cases. Natural polysaccharides have been particularly appealing in the development of new hemostatic materials due to their unique functions in human bodies. In this work, different polysaccharide-based hemostatic porous sponges (SHDP or SHDQ) of cross-linked hyaluronic acid (HA)/cationized dextran were readily prepared by the self-foaming process of HA and poly((2-dimethyl amino)-ethyl methacrylate)-grafted dextran (Dex-PDM) or partially-quaternized Dex-PDM in the presence of sodium trimetaphosphate crosslinkers. SHDP and SHDQ sponges were investigated in terms of liquid-absorption ability, hemolysis, whole-blood clotting and hemostatic activity in hemorrhaging-liver models. Compared with HA/Dex-PDM sponges (HDP) without chemical cross-linking, SHDP and SHDQ sponges displayed higher porosity (> 70.0% vs. 48.9%) and swelling ratios (> 1000% vs. 520%). Meanwhile, hemolysis assay revealed the good blood compatibility of SHDP and SHDQ with low hemolysis ratio (below 0.5%). Furthermore, *in vitro* and *in vivo* hemostatic assay showed that SHDQ possessed better hemostatic properties than SHDP, owing to the higher cationic charges of partially-quaternized Dex-QPDM than Dex-PDM. The present study demonstrated that the self-foaming process of HA/Dex-PDM under a 'green' condition is an effective means to produce new hemostatic materials.

1. Introduction

Bleeding stop is a crucial step of the emergency treatment, especially in cases of uncontrolled trauma in major traffic accidents, natural disasters and battle fields [1–3]. Hemorrhage (massive blood loss) normally cannot be controlled by the human body's natural hemostatic mechanism [4]. As a major supplement to traditional surgical techniques, a series of hemostatic materials have been explored for the treatment of hemorrhage in preclinical research and clinical trials [5–7]. In comparison with synthetic polymers and inorganic materials such as cyanoacrylates and zeolite, natural materials including fibrin glue, gelatin, and chitosan have been particularly appealing in the development of hemostatic materials due to their bioactive and unique biochemical functions in animal and human bodies [8–11].

Among natural materials, cationic chitosan has been recognized as the optimal one to construct hemostatic materials [11–14]. Amino groups of chitosan have the ability to interact with the negatively charged membranes of blood cells and thereby induce platelet aggregation, thus endowing chitosan with excellent hemostatic property [13]. However, the application of chitosan is limited by its poor water-solubility in physiological condition. Acetic acid is usually used as the processing solvent of chitosan, but it leads to a trace acidulous odor in the final hemostatic product [14]. Recently, cationic molecules and polycations had been introduced onto nonionic polysaccharides to construct cationized polysaccharides [15–18]. They could realize the interaction with negatively charged nucleic acids/cell membranes. Moreover, water-solubility of cationized polysaccharides is better than chitosan in physiological condition. Hence, it is very useful to explore the feasibility of cationized polysaccharides for effective hemostatic materials.

Hydrogels and sponges are the common product forms of hemostatic materials [12,19–21] Hydrogels are recognized as ideal injectable

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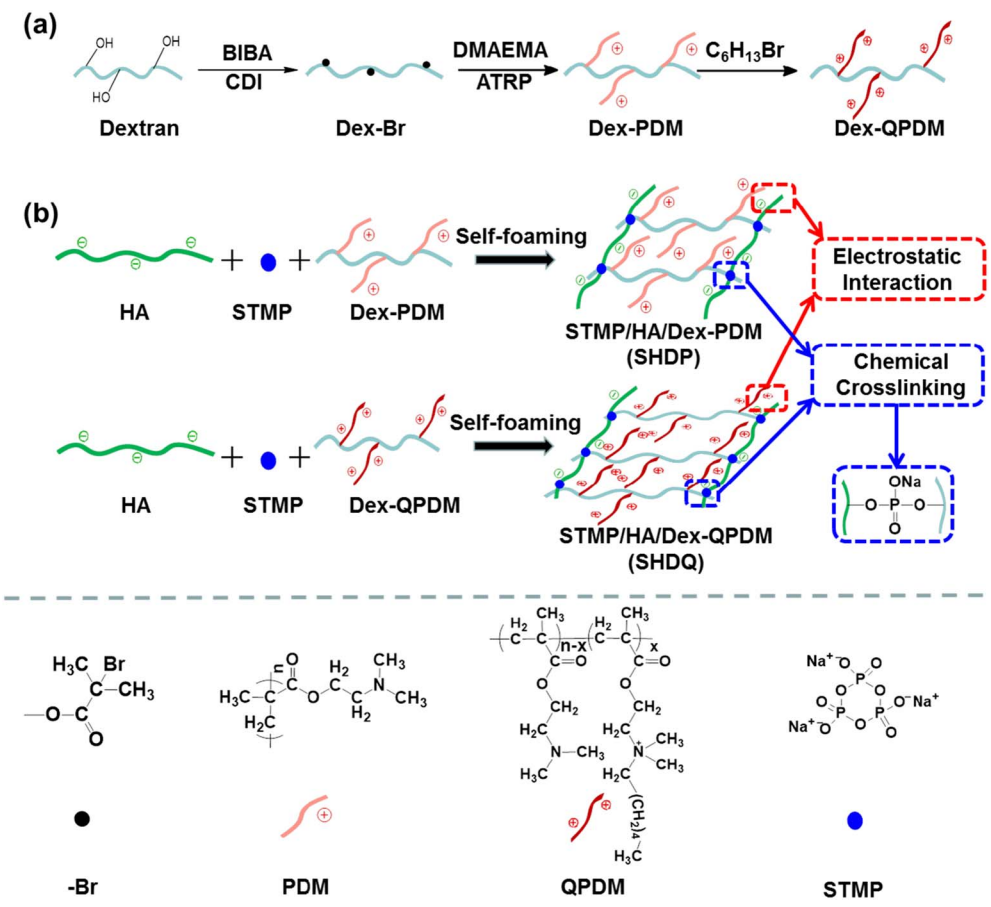

Fig. 1. Schematic illustration of the preparation of (a) cationized dextran and (b) hemostatic sponges.

biomaterials. Nevertheless, several basic requirements, such as rapid in-situ forming, mild crosslinking condition and enough mechanical strength, hinder the development of hydrogels for hemostatic applications [12,19]. Sponges can absorb wound exudate and blood from wound surface, concentrate blood cells/platelets and realize blood bleeding stop [20]. High adsorption property is prerequisite for high-performance hemostatic sponges. Sponges with highly porous structures exhibited high swelling ratios [21,22].

Herein, a simple self-foaming method was used to produce polysaccharide-based hemostatic porous sponges composed of hyaluronic acid (HA) and cationized dextran (Dex-PDM) (Fig. 1). A mixture of HA and Dex-PDM produced self-foaming solution under vigorous stirring without the addition of foaming agent (a 'green' condition). The electrostatic interaction between Dex-PDM and negatively charged HA served as physical crosslinking. HA/Dex-PDM foaming solution was also easily chemically cross-linked by sodium trimetaphosphate (STMP) to produce STMP/HA/Dex-PDM sponge (SHDP). In order to increase the positive charge concentrations of cationized dextran, the PDM side chains were partially quaternized to produce Dex-QPDM for the resultant STMP/HA/Dex-QPDM sponge (SHDQ). Such two types of hemostatic porous sponges were investigated in detail in terms of liquid-adsorption property, hemolysis rate, whole-blood clotting and in vivo hemostatic activity.

2. Materials and methods

2.1. Materials

Hyaluronic acid (200000–400,000 g/mol) was purchased from Bloomage Freda Biopharm CO., LTD. Dextran from Leuconostoc spp. (15000–25,000 g/mol), α-bromoisobutyric acid (BIBA, 98%), 1,1′-carbonyldiimidazole (CDI, 97%), copper(I) bromide (CuBr, 99%), *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%), sodium trimetaphosphate (STMP), bromohexane and (2-dimethylamino)ethyl methacrylate (DMAEMA, > 98%) were purchased from Sigma-Aldrich Chemical Co., St. Louis., DMAEMA was used after removal of the inhibitors in a ready-to-use disposable inhibitor-removal column (Sigma-Aldrich).

2.2. Preparation of cationized dextran (Dex-PDM) via ATRP

Some hydroxyl groups of dextran were reacted with BIBA (catalyzed by CDI) to prepare bromoisobutyryl-conjugated dextran (Dex-Br). Details on the preparation and characterization of Dex-Br were described in our previous work [17]. As shown in Fig. 1, the *comb*-shaped polymer (Dex-PDM) consisting of one dextran backbone and poly-DMAEMA (or PDM) side chains were prepared via atom transfer radical polymerization (ATRP) using Dex-Br as the macromolecular initiator. The typical ATRP conditions were used [17]. Briefly, a molar feed ratio [DMAEMA (4 mL)]: [CuBr]:[PMDETA] of 250:1.2:1.2 was used in a 8 mL methanol/water mixture (*v*/v = 2/6) containing 0.2 g of Dex-Br. The ATRP reaction was carried out at 30 °C for 0.5 h. The reaction mixture was diluted with water, then dialyzed against DI water (with dialysis bag of MWCO 3500) for 2 days and finally lyophilized.

2.3. Preparation of partially-quaternized Dex-PDM (Dex-QPDM)

Some tertiary amine groups of Dex-PDM were reacted with bromohexane, producing partially-quaternized Dex-PDM (Dex-QPDM) [17]. Dex-PDM (0.3 g) was introduced into the flask containing 10 mL of anhydrous dimethyl formamide. Then, different amounts (60, 80 or 100 μL) of bromohexane were added into the Dex-PDM solution and degassed with nitrogen. Finally, the flask was sealed with a rubber stopper and then kept stirring at 80 °C for 24 h. The final mixture was