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Preparation and anticoagulant properties of heparin-like electrospun membranes from carboxymethyl chitosan and bacterial cellulose sulfate

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ABSTRACT

Heparin-like membranes (CPBS) with nanofibers (approximate diameters of 100–500 nm) were prepared through electrospinning of a blended solution of carboxymethyl chitosan nanoparticle (CMCN, diameters 483 nm) and poly (vinyl alcohol) (CMCN/PVA) onto the surface of modified bacterial cellulose sulfate (BCS) membranes. SEM images confirmed that the CMCN were stretched to nanofibers during electrospinning. The presence of BCS on the collector of electrospinning machine increased the spinnability of CMCN/PVA solution. FTIR and XPS measurement revealed that there were $—SO_3^-$, COO^-, and —OH groups on the surface of CPBS membrane, expressing structural similarity to heparin. CPBS membranes maintained hydrophilicity and the glutaraldehyde crosslinked CPBS membrane was stable in water. The clotting time and platelet adhesion experiments expressed the anticoagulant properties of CPBS. The APTT, TT and PT of CPBS increased up to 116.0%, 189.8%, and 50% than those of the plasma, (67.4 s, 16.2 s, and 48.4 s, respectively). No platelets adhered onto the surface of CPBS. An inflammatory response was determined according to activation of the macrophages seeded onto the membranes.

1. Introduction

With the advent of technology-driven medical advances, many different biomaterials including metallic components, polymers, ceramics, and composite materials have been utilized for a variety of clinical applications. These materials have evolved over time in order to address unmet clinical needs either at the diagnostic or therapeutic fronts [1]. The characteristics of synthetic and natural products that are utilized in clinical scenarios where they come into contact with blood are pivotal to the outcome of a graft or implant. Physiologically acceptable blood/biomaterial compatibility is crucial for the successful application of blood-contacting biomaterials. During the blood clotting cascade, plasma proteins (mainly serum albumin, globulin, fibrinogen, and prothrombin) are rapidly absorbed onto the material surface in the presence of a foreign body. The adsorbed proteins activate platelets and induce platelet adhesion and aggregation, leading to blood clotting [2]. The characteristics of material surfaces (including chemical components, morphology, surface topography, surface charge, and surface wettability) are usually designed to modulate the process of protein adsorption and platelet activation. Heparin is a naturally occurring anticoagulant, which is a mixture of sulfated polysaccharide chains composed of repeating units of D-glucosamine and either L-iduronic or D-glucuronic acids [3]. The ionic functional groups including sulfate, sulfamide and carboxylate groups are the key groups of anticoagulant activity of heparin [4,5]. Heparin has been immobilized onto material to increase the blood compatibility through physical absorption or ionic bonding [6,7]. However, the immobilized heparin is easily released from the material and enters the blood, leading to abnormal blood flow and can contribute to subsequent tissue hemorrhage. Therefore, many alternative anticoagulant materials have been prepared through the introduction of specific functional groups onto the surface of blood-contacting material in order to mimic the structure of heparin through chemical grafting, plasma deposition, self-assembly [8–11]. Moreover, materials exhibiting specific design surfaces, especially those with nanofibrous membranes having similar chemical structures and nanofibers with the native extracellular matrix (ECM, including proteoglycans and fibrous proteins) and ranging from 50 to 150 nm in diameter accompanied by similar microtopography features (roughness, curvature and geometrical figures) with the blood vessel wall demonstrate good blood compatibility [12]. Therefore, a nanofibrous membrane that can mimic the structure and function of heparin may be beneficial with regards to blood compatibility.

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Electrospinning is an effective methodology for preparing nanofibrous materials with specific chemical structure and morphology through adjusting the polymer properties, electrospinning parameters, and electrospinning modes [13]. Electrospun materials have been used to mimic the surface of blood vessel walls, including chemical structure and morphology. Chitosan is a linear polysaccharide composed of randomly distributed β (1 → 4)-linked D glucosamine (usually >60%) and *N* acetyl D glucosamine. Chitosan is produced from deacetylation of chitin which is the only basic natural polysaccharide and whose total annual production is about 1.5 million tons available for commercial application, which guarantees the abundant resources of chitosan utilization. [14]. Chitosan has been widely investigated as a potential biomaterial due to its biocompatibility, biodegradability, and multiple biological properties [15]. It has been demonstrated that certain chitosan derivatives expressing negatively charged $—SO_3^-$ or —COONa groups exhibit enhanced blood compatibility due to the presence of sulfate, sulfamide or carboxylate groups [16]. Electrospinning of chitosan and carboxymethyl chitosan (CMC) has been investigated in recent years [15,17]. Owing to the strong molecular interaction between the highly charged density and high viscosity of chitosan and CMC solutions, it is very difficult to electrospin either chitosan or CMC [18,19]. Other polymers which have flexible polymer chains and can come into contact with chitosan through hydrogen bonds, including poly (lactic acid), poly (vinyl alcohol), polyethylene oxide, have been used to facilitate the electrospinning of chitosan and chitosan derivatives [20,21]. Typically, chitosan or its derivatives is first dissolved into a specific solvent and blended together with the polymer solution prior to electrospinning as this process is affected by polymer dissolution. Novel methodologies are currently being developed for the electrospinning of chitosan and its derivatives.

Bacterial cellulose (BC) is a type of natural cellulose produced by specific types of bacteria including *Acetobacter*, *Azotobacter*, *Rhizobium*, *Pseudomonas*, *Salmonella*, *Alcaligenes*, and *Sarcinaventriculi* [22]. BC differs from plant cellulose as it possesses unique structural properties, such as high chemical purity (cellulose content >98%), a high Young's modulus (up to 15 GPa), a high water-absorption capacity, and an ultrafine, reticulated fiber-like structure [23]. BC has been widely used in blood-contacting materials, such as artificial blood vessels, artificial skin, and wound healing materials. Cellulose sulfate, a half ester of cellulose, is widely studied in biotechnology and pharmaceuticals owing to its biological activities, such as anticoagulant, antibacterial, and antioxidant activity [24]. BC sulfate (BCS) can be utilized as a potential blood-contacting biomaterial owing to the presence of $—SO_3^-$, the mechanical property, and natural nanofibrous structure of BC.

Heparin possesses anticoagulation properties due to its interactions with specific proteins, especially antithrombin, which are mediated by the presence of functional groups on the polysaccharide chain (including $—SO_3^-$, $—COO^-$, $—NHSO_3^-$ and —OH) and the spatial orientation of the heparin polymer [25,26]. Nanofibrous membranes with specific chemical and morphological features express good blood biocompatibility. A nanofibrous membrane with sulfate, sulfamide, and carboxylate groups on the surface should have good blood compatibility. To obtain a heparin-like nanofibrous membrane, electrospinning of CMC and BCS together would provide a solid foundation for a blood biocompatibility biomaterial. CMC and BCS are negatively charged polymers and it is possible to blend CMC and BCS during electrospinning. Therefore, a novel electrospinning method was created and applied to fabricate a heparin-like nanofibrous membrane. CMC was first ball-milled to nanoparticle and then mixed with PVA solution to prepare electrospinning blend (CMCN/PVA). CMCN/PVA was then electrospun onto a BCS membrane attached to the collector. A heparin-like nanofibrous membrane (CPBS) with $—SO_3^-$, $—COO^-$, and $—NH_2$ on the surface was then obtained as depicted in Fig. 1.

2. Experimental

2.1. Materials

CMC (MW 3.4×10^5 g/mol, degree of deacetylation > 90%, particle size 2.9 μm) was purchased from HeFei Bomei Biotechnology Co., Ltd.

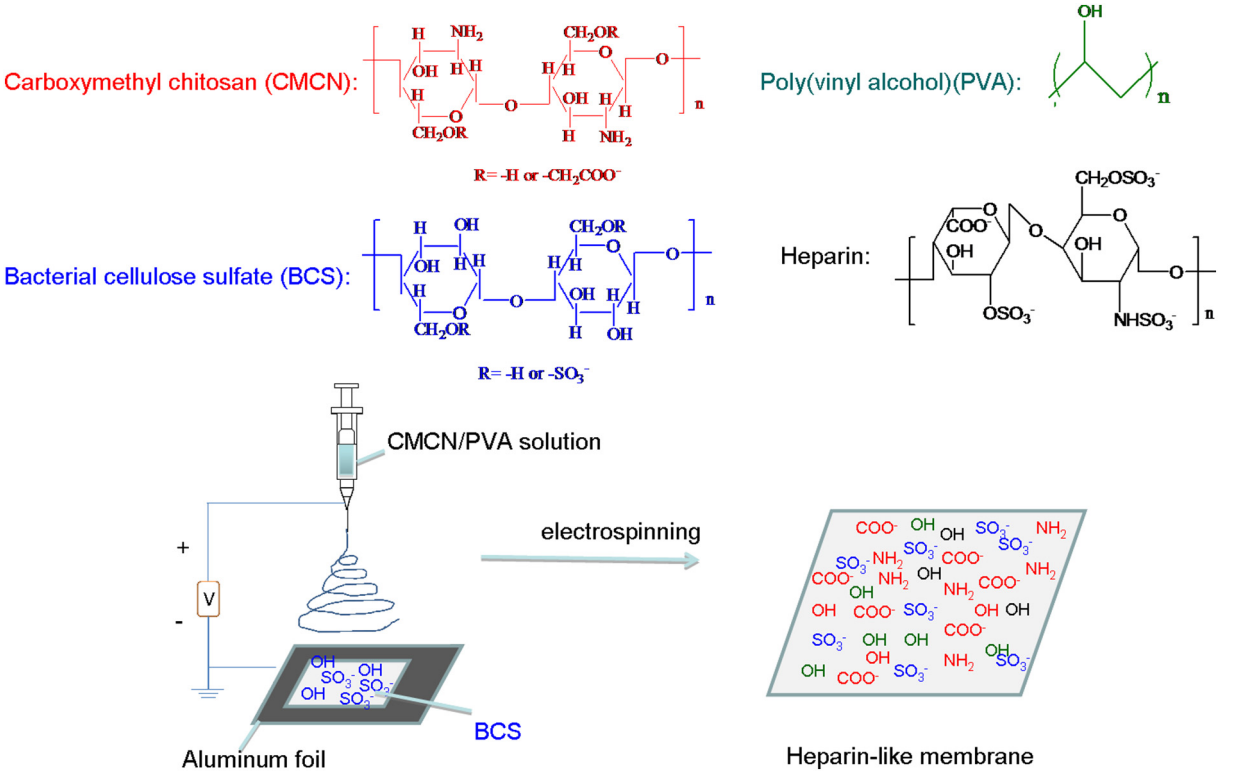

Fig. 1. Preparation Scheme of heparin-like membrane from carboxymethyl chitosan nanopowder and bacterial cellulose sulfate.