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Facile fabrication of bactericidal and antifouling switchable chitosan wound dressing through a 'click'-type interfacial reaction

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ABSTRACT

A facile approach to functionalize chitosan (CS) non-woven surface with the bactericidal and antifouling switchable moieties is presented. Azlactone-cationic carboxybetaine ester copolymer was firstly prepared, then chemically attached onto CS non-woven surface through the fast and efficient 'click'-type interfacial reaction between CS primary amines and azlactone moieties. The CS non-woven surface functionalized with cationic carboxybetaine esters is able to kill bacteria effectively. Upon the hydrolysis of carboxybetaine esters into zwitterionic groups, the resulting zwitterionic surface can further prevent the attachment of proteins, platelets, erythrocytes and bacteria. This CS non-woven that switches from bactericidal performance during storage to antifouling property before its service has great potential in wound dressing applications.

1. Introduction

Surface modification has been a significant issue over the past several years in many fields of application, especially biomedical field [1]. Several surface modification techniques have been developed to date, including plasma modification [2], UV irradiation [3], surface-initiated controlled graft polymerization [4], self-assembled monolayer (SAM) method [5], Langmuir–Blodgett (LB) technique [6,7] and layer-by-layer (LbL) assembly [8–10].

Chitosan (CS), a natural polysaccharide obtained by deacetylation higher than 50% of chitin, is a copolymer consisted of randomly distributed β-(1,4)-linked *N*-acetyl-D-glucosamine and D-glucosamine [11]. It is considered to be one of the most effective material for wound dressing mainly because of its biocompatibility, biodegradability and non-toxicity together with its antimicrobial activity and low immunogenicity [12]. Although CS has antimicrobial activity, its antimicrobial efficacy is influenced by several factors, e.g., deacetylation degree, ionic concentration, pH, bacterial species, etc. [13]. It has been confirmed that CS exhibits low antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* at physiological pH [14,15]. As for wound dressing, bacterial contamination and associated risk of infection is one of the most serious complications because they will cause the deterioration of wound, surgical failure, in severe cases, death [16]. Thus, the limited antimicrobial property of CS cannot meet the requirements of wound dressing.

Generally, there are two common approaches to impart antibacterial property to a material [17,18]. One approach is an "attacking" method which utilizes a wide range of bactericides or drugs including but not limited to silver ions [19], cationic polymers [20], antimicrobial peptides [21], and antibiotics [22] to actively kill bacteria. However, drug resistance or poor biocompatibility is of great concern [23]. The other approach is a "defending" method which uses antifouling coatings, e.g., poly(ethylene glycol) [24], poly(acrylamide)s [25], poly(*N*-vinylpyrrolidone) [26], poly(oxazoline) [27], and zwitterionic materials [28,29]. While the key deficiency of this bacteriostatic method is that this passive coating cannot kill bacteria or inhibit them proliferation once they attached on the surface [30]. Recently, the combination strategy of active bactericidal functions and passive antifouling property into a surface has been proposed [31]. Jiang et al. developed a novel switchable polymer surface that integrated bactericidal and antifouling properties [32–35]. In which, the bactericidal function

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was provided by quaternary ammonium moieties in the carboxybetaine ester polymers. The antifouling property was obtained by hydrolyzing from cationic carboxybetaine ester to zwitterionic groups.

At present, "attacking" method was mainly adopted to improve the antibacterial property of CS, i.e., physically loading and releasing bactericide [36,37], chemically immobilizing bactericide for sterilizing on contact [38–40]. As mentioned above, both good antibacterial efficacy and good biocompatibility cannot meet simultaneously. Recently, CS was modified with antifouling poly(ethylene glycol) and cationic charge through quaternization of the amino group, which presented good contact-active antibacterial performance and in vitro and in vivo biocompatibility [41].

Azlactones are lactone-based functional groups that react rapidly, quantitatively, and in the absence of a leaving group, with a broad range of functional nucleophiles through the fast and efficient 'click'-type reactions [42–45]. Due to the general versatility and potential practical advantages of polymers functionalized with azlactone moieties, they have been broadly used to design reactive surfaces and interfaces for the introduction of chemical and biological functionality [46–48]. As mentioned above, CS has a large number of primary amines, thus a simple and straightforward method can be conducted through the azlactone-containing copolymers. In this article, we proposed a facile approach to functionalize CS non-woven surface with the bactericidal and antifouling switchable moieties that made use of the 'click'-type reactions between primary amines in CS backbone and azlactone groups in azlactone-carboxybetaine ester copolymer. The in vitro biological response of the as-prepared CS non-woven to bacteria, protein, platelet and erythrocyte, was systematically investigated.

2. Material and methods

2.1. Material

Chitosan non-woven (CS) (degree of deacetylation ≥95%, and an average pore diameter of 100 μm) was supplied by Hismer Bio-Technology Co., Ltd. (China). 2-Vinyl-4,4-dimethylazlactone (VDMA) was obtained from Modapharm Shanghai Co., Ltd. (China). 2-Chloroethyl acrylate (CEA) and methyl 3-(dimethylamino) propionate (MDAP) and 2,2′-azobis(isobutyronitrile) (AIBN, recrystallized with methanol before use) were purchased from Sigma–Aldrich. Bovine serum fibrinogen (BFg; pI = 5.6), sodium dodecyl sulfate (SDS), phosphate buffered solution (PBS; 0.1 mol/L, pH 7.4), gram-negative *E. coli* (ATCC 25922), Luria-Bertani (LB) broth, and trypticase soy broth (TSB) were provided by Dingguo Biotechnology (China). Micro BCA protein assay reagent kit (AR1110) was purchased from Boster Biological Technology Co., Ltd. (China). Ethanol, methanol, toluene, dimethyl sulphoxide (DMSO), tetrahydrofuran (THF), *n*-hexane and acetonitrile were purchased from Haodi Chemical Reagents Co., Ltd. (China). The other reagents were AR grade and used without further purification.

2.2. Synthesis and quaternization of poly[(2-vinyl-4,4-dimethylazlactone)-co-(2-chloroethyl acrylate)] (p(VDMA-co-CEA))

Preparation of p(VDMA-*co*-CEA) was conducted in a 50 mL two-necked round flask with a condenser under argon. VDMA (8 mmol), CEA (16 mmol) and AIBN (0.16 mmol) were dissolved in toluene (15 mL). The solution was heated to 60 °C and stirred for 24 h. Then the copolymer solution was diluted with THF and precipitated into 7-fold excess chilled *n*-hexane under stirring to remove the unreacted monomer. The filtered copolymer p(VDMA-*co*-CEA) was finally dried under vacuum at 30 °C for 12 h and analyzed by ^{1}H NMR, ATR-FTIR and GPC.

Quaternization of p(VDMA-*co*-CEA) was conducted as follows. MDAP (2500 μL) and p(VDMA-*co*-CEA) (0.5 g) were dissolved into acetonitrile (1000 μL) at 60 °C for 24 h. Then the mixture solution was dissolved in DMSO, and precipitated into a large amount of chilled *n*-hexane under stirring. The obtained quaternization of p(VDMA-*co*-CEA) (Q-p(VDMA-*co*-CEA)) was dried at 30 °C for 12 h and analyzed by ^{1}H NMR and ATR-FTIR.

2.3. Modification of CS non-woven

The dried CS non-woven was immersed into DMSO solution (1000 μL) containing Q-p(VDMA-*co*-CEA) (0.1 g) for 24 h at 60 °C. After rinsing with DMSO and ethanol to remove physically adsorbed Q-p(VDMA-*co*-CEA), the Q-p(VDMA-*co*-CEA) modified CS non-woven (Q-CS) were dried overnight in a vacuum oven at room temperature.

2.4. Zwitteration of Q-p(VDMA-co-CEA) modified CS non-woven

The Q-CS non-woven was immersed into the mixed solution (pH 11.8) of 1.0 M NaOH and methanol at 37 °C. After 10 h of hydrolysis, the obtained zwitterionic CS non-woven (Z-CS) was washed by deionized water and ethanol, dried by vacuum.

2.5. Surface characterization

The ^{1}H NMR spectra was collected on a Bruker AV 400 M NMR spectrometer. All spectra were obtained in dimethyl sulfoxide-d_6 ($(CD_3)_2SO$) with tetramethylsilane as the internal standard at room temperature. Molecular weight of p(VDMA-*co*-CEA) was determined by using a gel permeation chromatography (GPC, Waters 410) at 35 °C with *N*,*N*-dimethylformamide (DMF) as elution solvent. ATR-FTIR spectra of the samples were obtained from a Fourier transform infrared spectrometer (FTIR, BRUKER Vertex 70) with a resolution of 4 cm^{-1} in absorbance mode. Static water contact angles (WCAs) of the samples were tested using a contact angle goniometer (DSA KRUSS GMBH, Hamburg 100) at room temperature by injecting 3 μL distilled water on the surface. Five measurements were conducted on each sample to calculate the average WCA values.

2.6. Nonspecific protein adsorption test

After equilibration by PBS overnight, the samples were immersed into single protein solutions of BFg (1.0 mg/mL) at 37 °C for 2 h. After rinsing several times with fresh PBS, the samples were soaked in an aqueous solution of 1.0 wt% SDS (1 mL), and the protein adsorbed on the surface was completely desorbed by sonication for 30 min. Based on the bicinchoninic acid (BCA) protein assay kit, the concentration of the protein in the SDS solution determined using the microplate reader (TECAN SUNRISE, Swiss) operating at 562 nm. Each result was an average of at least three parallel results. The results were expressed as the percentage of the amount of protein adsorption on the modified samples relative to that on CS.

2.7. Evaluation of platelet and erythrocyte adhesion

The samples were equilibrated with PBS solution overnight. Fresh blood extracted from a healthy rabbit in accordance with the guidelines issued by the ethical committee of the Chinese Academy of Sciences. The blood was centrifuged at 1000 rpm for 15 min to obtain the platelet-rich plasma (PRP) and erythrocyte concentrates. The erythrocyte concentrates were washed three times with PBS. Afterward, the erythrocytes were resuspended in PBS to obtain