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Protocols

Synthesis and characterization of incorporating mussel mimetic moieties into photoactive hydrogel adhesive

Zhiwen Zeng[a], Hongsheng Wang[c], Yosry Morsi[b], Xiumei Mo[a,c,d,*]

[a] State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Materials Science and Engineering, Donghua University, Shanghai 201620, China
[b] Faculty of Engineering and Industrial Sciences, Engineering and Technology, Swinburne University of Technology, P.O. Box 218, Hawthorn, Vicoria 3122, Australia
[c] College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China
[d] Shandong International Biotechnology Park Development Co, Ltd, 39, Keji Avenue, Yantai High-Tech Zone 264670, Shandong Province, China

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ABSTRACT

Surgical adhesive is the optimal candidate for the replacement of traditional mechanical wound closure. In our paper, mussel adhesive proteins inspired hydrogel adhesive was prepared with 3, 4-dihydroxyphyenylanine acrylamide (DOPA-AA), poly (ethylene glycol) diacrylate (PEGDAA) and thiolated chitosan (CSS) by UV irradiation. DOPA-AA, containing catechol group and vinyl group, was successful synthesized and characterized by FTIR and ^{1}HNMR. The gelation time, equilibrium water content, degradation, materials properties and adhesive strength of the hydrogels were studied. We found that the gelation time was retarded and the materials mechanical strength was decreased because of the inhibitory effect of catechol group. Equilibrium water content was slightly affect by the increasing concentration of DOPA-AA (1–5%). Nevertheless, catechol group can remarkably improve the adhesive properties because of the complex and durable interactions of the hydrogel, especially, the interaction between the thiol group of CSS and catechol group of DOPA-AA, which also greatly slowed down the degradation of the adhesive hydrogels. CSS and DOPA-AA was introduced to ensure the adhesive properties, DOPA-AA lend the adhesive nature to hydrogel and CSS can protect the catechol group from oxidation and enhance durable adhesion. Moreover, cytotoxicity of the resulting hydrogels showed that the L929 cell viability was weaken, it mostly probably induced by the catechol oxidation.

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1. Introduction

Bioadhesives have increasingly gained its attraction and popularity as novel biomaterials because of their obviously advantages including rapid control bleeding and bonding, less pain, no second surgical risking and minimum infection [1,2]. Therefore, it universal utilized for surgical adhesives, hemostatic agents, wound closing, dental bonding, and so on [3]. Tissue adhesives can effectively deal with the problems that the traditional mechanical wound closure devices (such as sutures, staples, tacks) can not, for example, traditional wound closure devices would create new trauma and unable to reconnect soft tissues with low cohesive properties (e.g., dura, kidney, lung, spleen) [2,4,5]. Nevertheless, Tissue adhesives have to address the two main problems: (1) the adhesion strength is not enough strong to integrated with the local tissue and the durable adhesion is lost due to the presence of body fluids; (2) adhesives or its degradation products is not biocompatible. Existing commercial tissue adhesives, such as fibrin sealant and cyanoacrylate, are hampered by these two problems. Therefore, a biocompatible tissue adhesive with superior adhesion performance is highly desirable and urgent to develop and promote.

Marine mussels can attach to various matrix surfaces tightly under wet and salty environment by secreting exceptional underwater adhesive proteins, which was named of mussel adhesive proteins (MAPs) [6,7]. MAPs contains a high content of the unusual unique amino acid 3, 4-dihydroxyphyenylanine (DOPA), which believed to lend outstanding adhesive performance to the MAPs [8]. Waite group [9,10] identified that the catechol group contained in DOPA is mainly responsible for the high, strong moisture-resistant adhesion of MAPs. Catechol group is a reactive, sensitive DOPA residue and easily oxidized by chemical or enzymatic to form DOPA-quinone that rapidly reacts with basic amino acids or nucleophilic groups. Thus, modification of DOPA and its derivatives

* Corresponding author at: College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China.
E-mail address: xmm@dhu.edu.cn (X. Mo).

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onto bioadhesive materials have a great of potential application in clinical medicine as surgical adhesive and tissue cement [2,4,11]. Several literatures suggested that un-oxidized DOPA is responsible for strong water resistant adhesion while the oxidized *o*-quinone is responsible for cross-link formation [12]. Therefore, to protect the catechol group, scientific researchers recently begin to synthesis DOPA-containing biomimetic polymers that cross-linked to form hydrogels quickly without oxidizing reagents. Some strategies are proposed to address the challenge, such as preparation of thermosensitive materials grafted with DOPA residues [13–15], incorporating DOPA or its derivatives to polymers that can cured by photopolymerization, [16,17] or metal ions [18,19], and so on. Besides, polymers containing DOPA or analogous catecholic moieties have also designed to form hydrogels via chemical cross-linking with polymers containing active groups [20].

However, the facile tendency toward auto-oxidation of DOPA-containing biomimetic polymers have to take into consideration when it was dissolved in the aqueous solution, which usually renders DOPA unreliable for adhesion. Jing Yu et al. [21] showed an interesting findings that Mussels limit DOPA oxidation by imposing an acidic reducing regime based on thiol-rich mfp-6, which restores DOPA by coupling the oxidation of thiols to dopaquinone reduction. Therefore, in this paper, in order to make use of the adhesive characteristics of DOPA and protect the catechol group from auto-oxidation, we have designed an adhesive composed of three polymers, namely, 3, 4-dihydroxyphyenylanine acrylamide (DOPA-AA), poly (ethylene glycol) –diacrylate (PEGDAA) and thiolated chitosan (CSS). DOPA-AA, containing catechol group and vinyl group, was synthesized and its chemical structure was characterized by Fourier transform infrared spectra (FTIR) and proton nuclear magnetic resonance spectroscopy (^{1}HNMR). CSS was modified with thiol group that can react with vinyl group and restore catechol group. The copolymerization of DOPA-AA, PEGDAA and CSS was initiated in aqueous solution to form hydrogel by UV irradiation. The gelation time, equilibrium water content, degradation, materials properties, adhesive strength of the hydrogel were determined. Moreover, the cytotoxicity of the resulting hydrogel was also evaluated by MTT assay.

2. Experimental section

2.1. Materials

Chitosan used was purchased from Sigma-Aldrich, medium molecular weight, with an 85% nominal degree of deacetylation. 1-Hydroxybenzotrizole Hydrate (HOBT) was purchased from Shanghai WoKai chemical Reagent co Ltd. Poly (ethylene glycol) (PEG, 2 K Da) and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) were purchased from sinopharm chemical reagent Co Ltd. 3,4-dihydroxyphyenylanine (L-DOPA) was purchased from Sigma-Aldrich. Acryloyl Chloride was purchased from Energy Chemical. The photoinitiator, 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one, namely Irgacure 2959 (I2959) was purchased from Sigma-Aldrich. Fibrin sealant (Tisseel ®) was purchased from Baxter Healthcare Ltd, 3-[4,5-dimethylthiazol-2-yl]-2,5diphenyltetrazolium bromide (MTT) was purchased from Biosharp, Fetal bovine serum (FBS) and 0.25% Trypsin-EDTA was purchased from Gibco, Dulbecco's modified eagle medium (DMEM, high glucose) was purchased from Hyclone. Other chemical reagents were all purchased from Sinopharm Chemical Reagent Co., Ltd and were used as received.

2.2. Synthesis of L-DOPA (DOPA-AA)

The reaction media was prepared by dissolving 2.288 g (0.006 mmol) of sodium borate and 1.272 g (0.012 mmol) of sodium bicarbonate in 50 mL of distilled water, the resulting aqueous solution was degassed with N_2 for 30 min to remove the air from the solution to protect the catechol group. After 1.183 g (0.006 mmol) of L-DOPA was added and stirred for 20 min, the solution was cooled to 0 °C, 0.652 g (0.072 mmol) of acryloyl chloride in 5 mL of dichloromethane was prepared and added dropwise slowly into the aqueous solution with stirring. The reaction mixture solution was stirred for 24 h at room temperature with nitrogen bubbling meanwhile the pH of the reaction solution was maintained above 9.0 with 1 M of NaOH. The resulting reaction solution was acidified to pH 2 with concentrated HCl and then extracted with ethyl acetate for 3 times. The extracted solution was dried over anhydrous MgSO4, filtered and concentrated, then added to 200 mL hexane with vigorous stirring to precipitate a brownish solid. The formed suspension was refrigerated to maximize crystal formation size. After filtration, to purify, the obtained crude product was dissolved in ethyl acetate and precipitated in hexane again. After dried in a vaccum drying oven, the samples were dissolved in ultra-pure water, then lyophilized to afford the final powder product.

2.3. Synthesis of CSS and PEGDAA

Thiolated chitosan (CSS) and poly (ethylene glycol) –diacrylate (PEGDAA) were synthesized as the literature description [22]. Briefly, 0.5 g of chitosan powder was dispersed in 50 mL ultrapure water on a flask under stirring for 20 min. The solution became clear under stirring after HOBT (2.58 mmol) was added, and then NAC (5.16 mmol) was added to the mixture solution followed by the addition of a solution of EDC·HCl (10.32 mmol). The reaction was proceeded for 5 h at room temperature. The pH value of the solution was keeping at 4–5 by the addition of 1 M HCl solution during the reaction. After 5 h, the reaction solution was dialyzed and lyophilized. The lyophilized product was CSS, and content of thiol group was determined by Ellman's test. (^{1}HNMR Data was shown in Fig. S1).

8 wt% of PEG was dissolved in toluene on a flask then the moisture contained in the PEG was removed by azeotropic distillation using a Dean-Stark trap. After cooling to room temperature, the PEG solution was degased with N_2 for 30 min. Triethylamine (TEA) dissolved in dichloromethane (volume ratio = 1:9) was added to the PEG solution with stirring. The reaction begun with the acryloyl chloride added into the solution dropwise and proceeded for 24 h under N_2 atmosphere. The reaction solution was filtered and concentrated. Then PEGDAA was precipitated from the concentrated solution by addition of cooled diethyl ether. The precipitate was washed with diethyl ether for three times and then dried in vacuum drying oven at room temperature. The react molar ratio of the PEG: TEA: acryloyl chloride was 1:2:2. The substitution of the vinyl group was determined using the ^{1}HNMR spectrum. (^{1}HNMR Data was shown in Fig. S2).

2.4. Characterization of DOPA-AA monomer

2.4.1. FTIR spectra measurement

The resultant DOPA-AA was analyzed by FTIR spectra which recorded on Nicolet 6700 instrument (Thermo Fisher Company, USA) equipped with an attenuated Total Reflection (ATR) (Thermo Fisher Company, USA) attachment. The measurement was carried out fast at wavenumber from 4000 to 600 cm^{-1} with resolution of 4.0 cm^{-1} so that DOPA-AA was not oxidized to form DOPA-quinone. Besides, the FTIR spectroscopy of L-DOPA was also collected.

2.4.2. ^{1}HNMR spectroscopy of DOPA-AA

To confirm the successful grafting of acrylamide group to L-DOPA, DOPA-AA was also analyzed by ^{1}HNMR spectroscopy. 1.5% (g/mL) of DOPA-AA solution in deuterium oxide (D_2O) was trans-

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