Synthesis and Characterization of Rhodamine B-ethylenediamine-hyaluronan Acid as Potential Biological Functional Materials

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Abstract. The purpose of this study is to synthesize and characterize fluorescent polymers, rhodamine B-ethylenediamine-hyaluronan acid (RhB-EA-HA). RhB-EA-HA was successfully synthesized by ester ammonolysis reaction and amidation reaction. Moreover, the structural properties of RhB-EA-HA were characterized by 1H-NMR spectra, UV-vis spectrometry and Fourier transform infrared spectroscopy (FT-IR). RhB-EA-HA can be grafted on the surface of silica nanomaterials, which may be potential biological functional materials for drug delivery system.

1. Introduction
The rhodamine B (RhB) is a basic dye that belongs to xanthene which has been widely used in many fields owing to their high quantum yield, high absorption coefficient and excellent light stability [1-2]. It has been used as dye due to the bright color at the early stage. In the recent years, the use of RhB for fluorescent probes, especially in single-molecule imaging [3-4] and imaging in living cells [5-7] has also been reported. HA is an unusual polysaccharide that has a simple chemical structure but excellent properties with large, negatively charged, unbranched polymer which is composed of repeating disaccharides of glucuronic acid and N-acetylglucosamine:[β(1,4)-GlcUA-β(1,3)-GlcNAc-]n[8].It has been widely used for biomedical application owing to its excellent biocompatibility [9].Therefore, HA has attracted considerable attention as the targeting ligands of drug delivery systems owing to a specific interaction with CD44 receptors that are over expressed on various tumor cells [10].

The results showed that the number of cases diagnosed with cancer and surviving in China within 5 years was about 7.49 million (including 3.68 million male patients and 3.81 million female patients), the overall 5-year cancer prevalence was 556/10 million [11]. The traditional means of cancer treatment mainly for chemotherapy due to it can not distinguish between rapidly dividing normal cells and cancer cells therefore limit its application [12]. To overcome the limitations associated with nonselective chemotherapeutics, ‘smart’ drug delivery systems have been developed [13-14]. Mesoporous silica has been widely used for drug carrier and controlled release owing to its
stable porous structure, tunable pore sizes and volumes, large surface area, easier chemical functionalization of the surface, and excellent biocompatibility [15-16]. In our present work, the obtained results indicated that the RhB-EA-HA were successfully synthesized. Furthermore, in the further work, we will research the combination of mesoporous silica with RhB-EA-HA and further applied to drug delivery system. In this system, HA is not only act as capping agents but also as targeting ligands and RhB is used as fluorescent probes. Therefore, the RhB-EA-HA might be a great potential modified nanomaterials as a drug delivery system in the future.

2. Experimental section

2.1. Materials
Rhodamine B, Dichloromethane, Anhydrous ethanol, Ethylenediamine, Sodium hyaluronate (MW100KDa), Dialysis bag, Sodium sulfatede hydrochloric acid, Sodium hydroxide were obtained from Sinopharm Chemical Reagent Co. Ltd. 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimidehydrochloride (EDC·HCl) were purchased from J&K Chemical Co. (Beijing), All the chemicals were used as received without further purification.

2.2. Synthesis of RhB-EA
RhB reacts with EA were prepared according to the published method [17]. And the synthesis route was presented in scheme 1(A). Briefly, To a 250mL round-bottom flask, RhB (2.40g, 5mmol) was first dissolved in 60mL ethanol. 0.43mL of EA (6.5mmol) was then added dropwise with vigorous stirring at room temperature. After the addition, the stirred mixture was heated to reflux for 12h. The solution became clear. Then the mixture was cooled and solvent was removed under reduced pressure. 1M HCl (about 100mL) was added to the residue to remove any unreacted EA. After that, 1MNaOH (about 140mL) was added slowly with stirring until the pH of the solution reached 9–10. The resulting solution was extracted with dichloromethane (3*100mL), washed the organic layer with 50mL water, dried on Na₂SO₄ and evaporated the solvents to get the crude product.

2.3. Synthesis of RhB-EA-HA
The synthesis route was presented in scheme 1(B). HA (100mg) was dissolved in nanopure water (50ml) and then activated by using EDC (150mg) and HCL (100mg) for 1h at room temperature. Then an aqueous solution of RhB-EA as prepared (10.5mg) was added dropwise with avoiding light and stirring at room temperature for 24 h. And the mixture was dialyzed for two days then resulting an aqueous solution of RhB-EA-HA which was freeze-dried.

Scheme 1. Synthetic scheme of the RhB-EA-HA.
2.4. Characterizations
Fourier transform infrared (FT-IR) spectrum was noted on Nicolet Nexus 470. The UV spectrum was determined using a UV-Vis spectrophotometer (UV-2450PC, Shimadzu). \(^1\)H NMR spectra were recorded on a Bruker AM-400 NMR spectrometer in CD\(_3\)OD and D\(_2\)O.

3. Results and discussions
As shown in Fig. 1(A), HA is not absorbed in the UV region. In Fig. 1(B), RhB has a strong absorption peak at 554 nm due to the presence of aromatic heterocyclic ring. In Fig. 1(C), although the peak intensity of HA by rhodamine derivative (RhB-EA) modified was reduced, the absorption peak at 554 nm was observed, these results indicating that RhB had been successfully modified to HA.

The successful incorporation of RhB and EA to HA is further confirmed by FT-IR spectra. As shown in Fig. 2, compared with RhB Fig. 2(A), a new adsorption peak appeared at 1633 cm\(^{-1}\) in RhB-EA Fig. 2(B) and RhB-EA-HA Fig. 2(C), which is attributed to the amide bond stretching vibration of C=O peak. So, it indicated that the HA were successfully modified with the RhB and EA.

Figure 1. UV-Vis spectral of the HA(A), RhB(B), RhB-EA-HA(C).

Figure 2. The FT-IR spectra of RhB (A), RhB-EA(B) and RhB-EA-HA(C).

Figure 3. \(^1\)H-NMR spectrum of RhB-EA-HA.

The structures of RhB-EA-HA was also determined by \(^1\)H-NMR analysis (Fig. 3). As displayed in Fig. 3, the presence of peak at δ 2.00 corresponded to the protons of characteristic peak of N-acetyl
methyl of HA. And two peaks appeared at δ 1.26, δ 1.12 were attributed to the protons of the methylene peak of RhB derivates(RhB-EA). The presence of peak at δ 5.06 corresponded to the protons of methylene peak of HA. The red arrows in Fig. 3 indicate the characteristic peaks of the benzene ring of the RhB. These results suggested that RhB was successfully grafted onto the main chain of HA.

4. Summary
In this paper, the RhB-EA-HA was successfully synthesized. Moreover, the RhB-EA-HA were characterized by 1H-NMR, UV-Vis and FT-IR. These results would provide a potential biological functional materials for the modified mesoporous silica nanoparticles to applying in drug delivery system. So, in the next work, we will research the combination of mesoporous silica with RhB-EA-HA and further applied to drug delivery system. In this system, HA is not only act as capping agents but also as targeting ligands and RhB is used as imaging in living cells. So, the RhB-EA-HA would be a great potential modified nanomaterials as a drug delivery system in the future.

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