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Room temperature preparation of fluorescent starch nanoparticles from starch-dopamine conjugates and their biological applications

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ABSTRACT

Fluorescent organic nanoparticles (FONs) have been regarded as the promising candidates for biomedical applications owing to their well adjustment of chemical structure and optical properties and good biological properties. However, the preparation of FONs from the natural derived polymers has been rarely reported thus far. In current work, we reported a novel strategy for preparation of FONs based on the self-polymerization of starch-dopamine conjugates and polyethyleneimine in rather mild experimental conditions, including air atmosphere, aqueous solution, absent catalysts and at room temperature. The morphology, chemical structure and optical properties of the resultant starch-based FONs were investigated by different characterization techniques. Biological evaluation results demonstrated that these starch-based FONs possess good biocompatibility and fluorescent imaging performance. More importantly, the novel strategy might also be extended for the preparation of many other carbohydrate polymers based FONs with different structure and functions. Therefore, this work opens a new avenue for the preparation and biomedical applications of luminescent carbohydrate polymers.

1. Introduction

In recent years, fluorescent nanoprobes have drawn much attention and inspired the research enthusiasm as their high sensitivity, well designability and simple operation in practical applications [1]. Thus, they have been widely used in materials science, food science, chemical sensor, especially biological detection and recognition [2,3]. In view of the chemical compositions, fluorescent nanoparticles can be divided into three classes, including fluorescent proteins, luminescent inorganic nanomaterials and organic dyes based nanoparticles [4–15]. Green fluorescent proteins, red fluorescent proteins and other luminescent proteins have been reported in a large number of literatures since 1962, and became important tools of biological science [16–18]. But what cannot be ignored was the low membrane permeability, high cost and poor photostability. The second type, such as metal nanoclusters, semiconductor quantum dots, carbon nanodots and luminescent silicon nanoparticles, exhibited outstanding fluorescence property in most case [19–23]. However, they were suffered from the intrinsic drawbacks, such as non-biodegradable, high toxicity. Fluorescent conjugated polymers and small organic dyes and aggregation-induced emission (AIE) dyes based fluorescent organic nanoparticles (FONs) were the most emerging examples for fluorescent probes, which possessed high sensitivity, good biocompatibility and excellent designability [24–29]. However, one notable drawback of most fluorescent conjugated polymers and small organic dyes based FONs was aggregation-caused quenching (ACQ) effect. Although AIE-active dyes based FONs could overcome the ACQ effect of FONs based conventional organic molecules, these FONs should be first synthesis of AIE-active organic molecules and then incorporate these molecules into FONs through different strategies. Therefore, FONs based on organic molecules were devaluated by the expensive precursors and complex synthesis procedure [30–33]. In order to conquer the above shortcomings, developing novel strategies for preparation of FONs with better properties through low cost precursors under mild experimental conditions are still highly desirable.

Dopamine (DA), an important model molecule in mussel inspired chemistry, has attracted much great attention in recent years and has been applied in various fields [34–36]. It has been demonstrated that

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DA can self-polymerize into polydopamine (PDA) under alkaline aqueous environment. More importantly, PDA showed strong adhesion to different inorganic and organic matrices regardless of their morphology, size and structure [37–41]. In light of this, the self-polymerization of DA opened the applications from catalysis, environmental adsorption to biomedicine etc. [42–46]. In biomedicine, Liu et al. employed PDA coated super-paramagnetic iron oxide nanoparticles for photothermal cancer therapy [47]. On the other hand, Zhao et al. reported the layer of PDA can obviously improve the blood compatibility and biological activity of heparin-mimicking membranes [48,49]. Huang et al. also showed the unique advantage of PDA in the mechanical properties, stability and biocompatibility [50]. To date, only very few studies have reported the fabrication of FONs based on PDA. In this aspect, Wei et al. have suggested that PDA based FONs can be facilely obtained by the oxidation of PDA using concentrated H_2O_2 [51]. After that, Frank group reported the synthesis of fluorescent PDA capsules through the similar route and these luminescent capsules were utilized for biological imaging and drug delivery applications [52]. More recently, we have demonstrated that fluorescent polymers can be facilely formed by self-polymerization of DA and polyethyleneimine (PEI) in rather mild experimental conditions. These fluorescent PDA nanoparticles displayed strong fluorescence intensity, high water dispersibility and can be potentially used for biological imaging applications [53]. The self-polymerization of DA should be a simple, novel and useful strategy for preparation of FONs, however, there have no literatures were focused on the fabrication of fluorescent carbohydrate polymers thus far.

Starch has been generally explored as a precursor in spinning, papermaking, foodstuff and biomedical products in view of its low cost, native, degradability, stability and biocompatibility [54]. In biomedicine, starch and chitosan based hydrogels, starch-based FONs from hydrothermal treatment and starch-based FONs with AIE feature have been reported in succession [55,56]. In this paper, starch was chosen as the precursor to synthesize starch-based FONs. The detailed experimental procedures were shown in Scheme 1. First, the aldehyde groups were introduced onto starch through oxidized by $NaIO_4$. Then, DA was linked with the oxidized starch using mercaptoacetic acid locking imine (MALI) reaction. Finally, PEI was added to react with starch-DA conjugates to obtain the starch-based FONs. The resultant FONs were characterized by various techniques. The potential biomedical applications of starch-based FONs were further evaluated based on cell viability and cell uptake behavior.

2. Materials and methods

2.1. Materials and measurements

PEI (Mw = 600 Da, Aladdin reagent Inc. Shanghai, China), DA (Sangon Biotech, Shanghai, China) and starch from corn (Aladdin Reagent Inc. Shanghai, China) were used as received. All other solvents and chemicals were purchased from commercial sources and used directly without further purification. Fluorescence spectra were measured on a Hitachi FS F-4500 spectrometer with a slit width of 5 nm for both excitation and emission spectra. The ^{1}H nuclear magnetic spectroscopy was conducted on Bruker Avance-400 spectrometer. Transmission electron microscopy (TEM) images were recorded on a Hitachi 7650B microscope operated at 80 kV; the TEM specimens were obtained by placing a drop of ethanol nanoparticle suspension on a carbon-coated copper grid. The Fourier transform infrared (FT-IR) spectra were recorded in a transmission mode on a Nicolet 5700 spectrometer (Waltham, MA, USA). The dynamic size distribution of starch-based FONs in phosphate buffer saline (PBS) was determined by dynamic laser scattering (DLS) using ZetaPlus apparatus (Brookhaven Instruments, Holtsville, NY).

2.2. Preparation of dialdehyde starch

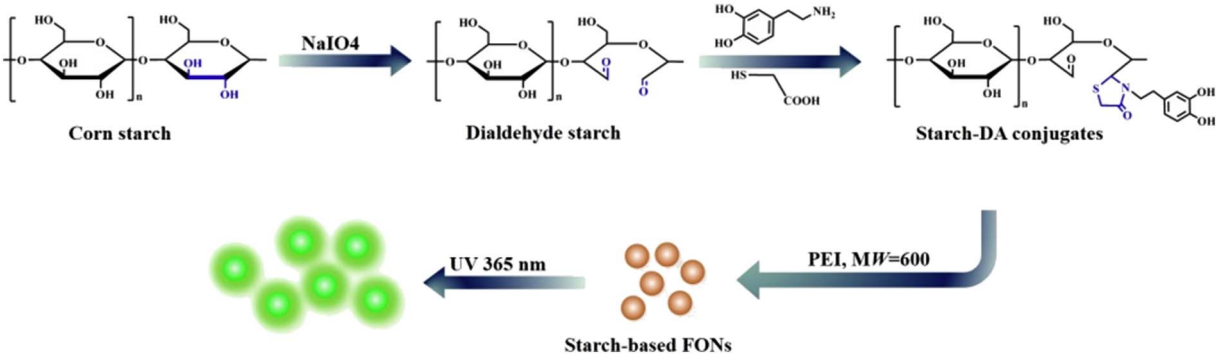
Starch oxidation adopted the modified method described by Christensen [57]. Briefly, 1 g corn starch was put into deionized water to form 6.0% W/V starch solution, and then sodium periodate in 1:2 equivalent molar ratio was added into the solution. With magnetic stirring, the system was maintained for 6 h in dark ambient under room temperature. After reaction, the dialdehyde starch can be obtained through filtration and washing repeatedly. Finally, the content of aldehyde group was calculated according to the consumption of alkali. Briefly, 0.1 g of dialdehyde starch was added into 2.5 mL of distilled water. The mixture was heated for 30 min at 40 °C. The pH values were adjusted to 5.0 after cooling down to room temperature. Then, hydroxylamine hydrochloride (1 M) solution (pH = 5.0) was added into the reaction mixture to form oxime moieties where an HCL equivalent for each aldehyde group was released. Using phenolphthalein as indicator, the mixture was titrated with 1 M NaOH. When the mixture began to turn red, the consumption of NaOH solution was recorded as V_a. The blank control test was conducted following the above and recorded V_b.

$$\text{Percentage of aldehyde content} = \frac{N_{\text{NaOH}} \times 0.028 \times (V_b - V_a) \times 100}{m}$$

N_{NaOH} and m represent 0.1 N and dry weight of dialdehyde starch, respectively.

2.3. Preparation of starch-based FONs

600 mg dialdehyde starch was first dissolved in dimethylsulfoxide (DMSO) under 45 °C. Do not stop heating until the mixture formed solution. After cooling down to room temperature, DA and mercaptoacetic acid in 1:1:1 equivalent molar ratio was added into the solution. Then the system was kept 2 h at room temperature. Finally, the starch-DA conjugates were obtained via dialysis procedure. The detailed process is shown in Scheme 1. In the next, starch-DA conjugates and PEI in 1:1.5 equivalent molar ratios were dissolved into deionized



Scheme 1. Reaction scheme for the preparation of starch-based FONs.