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Fabrication of stable and biocompatible red fluorescent glycopolymer nanoparticles for cellular imaging

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ABSTRACT

Glycosylated cross-linked red fluorescent amphiphilic polymer has been facilely synthesized in one pot, which would readily self-assemble into nanoparticles with high water dispersibility in aqueous media. The resulted nanoparticles have surplus carboxyl groups and glycosyl groups on the surface, which can be further functionalized, and they can demonstrate strong bio-favorable red fluorescence with a fluorescence quantum yield of 18% due to the aggregation induced emission (AIE) dyes aggregated in the core. Moreover, the nanoparticles also revealed great photostability and structure stability with an ultralow critical micelle concentration of 0.0018 mg mL^{-1}. Their excellent biocompatibility and cell uptake behavior make them promising for cellular imaging.

1. Introduction

Cellular fluorescence imaging has captured great attention as a versatile visualization way for medical diagnosis, drug development, and clinical study.[1–7] Therefore, various fluorescent bioprobes have been extensively studied over the past decades, such as fluorescent protein, semiconductor quantum dots, rare earth-doped upconversion nanoparticles, and organic dye-doped nanoparticles.[8–13] Among these diverse fluorescent probes, red emissive bioprobes were more attractive in the depth imaging and tracing applications, because the red fluorescence was far away from the bio-autofluorescence emission range and can penetrate deeper into the biology systems to obtain images with high contrast and resolution.[14–16] However, fluorescent protein often encounters the problem of poor photostability, small stokes shifts, and tedious transfection process.[17] While both inorganic semiconductor quantum dots and rare earth-doped upconversion nanoparticles suffer from the high cytotoxicity due to the containing of heavy metal ions, which severely restricts their real biomedical applications.[18–20] For most organic dyes-doped nanoparticles, the hydrophobic planar structures of dyes will induce strong intermolecular π−π interactions, leading to fluorescence quenching and photobleaching, which was especially serious for red fluorescent molecules.[21–23] To conquer this aggregation-caused quenching problem, another type of unique organic dyes were developed by Tang et al. in 2001, which can emit much stronger luminescence in their aggregation states and were called aggregation induced emission (AIE) dyes.[22,24] After that a large number of AIE fluorogens and these AIE fluorogens based fluorescent polymer nanoparticles have been rapidly developed and extensively investigated for chemosensors and bioimaging applications by now.[25–37] Nevertheless, as many aforementioned AIE based fluorescent polymers nanoparticles are formed by self-assembling of linear polymers, which are not stable in dilute solution below the critical micelle concentration (CMC).[38–40] In this case, cross-linked polymeric nanoparticles are expected to be more stable in the practical applications. Therefore, preparation of novel stable cross-linked fluorescent polymeric nanoparticles based on AIE dyes is of great scientific interest, and more versatile strategies are still highly demanded.[41]

In order to endow the AIE based polymer with favorable compatibility for bioapplications, biomass monomers seem to be very attractive for the construction of fluorescent polymer nanoparticles. Glucose is an ideal candidate, which is frequently utilized in the fabrication of biomedical polymers. For examples, glucose-containing polymers demonstrated huge potential for various

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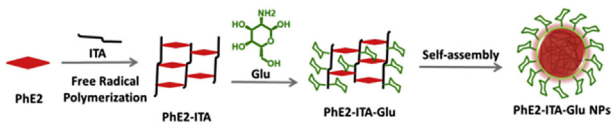
tissue engineering and biomedical applications, since they can be facilely functionalized as therapeutic drugs, drug delivery vehicles, cell culture matrices and biocompatible materials.[39,42–44] In addition, carbohydrates at the cell surface play important roles in diverse biological recognition events owning to the specific reactions of glucose with proteins and other biological entities (e.g., cells, pathogens).[45–48] In order to connect glucose monomer with AIE monomer, itaconic anhydride (ITA) is a good cross-linker since ITA can be facilely polymerized and is reactive with amide compounds. Moreover, ITA is also very economic and benign to environment. Therefore, by combining with these two biomass monomers together, fluorescent glycopolymer nanoparticles (FGNs) prepared using ITA and AIE monomers would become promising biomaterials for tracing and medical applications.

Herein, stable red florescent glycopolymer based on AIE dyes (**PhE2-ITA-Glu**) was facilely synthesized via free radical polymerization and ring-opening reaction from AIE monomer (**PhE2**), biomass monomer itaconic anhydride (**ITA**), and glucosamine hydrochloride (**Glu**) for the first time. The obtained **PhE2-ITA-Glu** amphiphilic polymer was readily self-assemble into stable nanoparticles owning to their cross-linked structures, and the surplus glycosyl groups covered on the surface of these FGNs endowed them with great water dispersibility. More importantly, the FGNs demonstrated intense bio-favorable red fluorescence with high fluorescence quantum yield and great photostability thanks to the red AIE dyes aggregated in the core. Furthermore, the biocompatibility and cell uptake behavior of **PhE2-ITA-Glu** FGNs were also determined to evaluate their potential cellular imaging applications (Scheme 1).



Scheme 1. Schematic showing the preparation of **PhE2-ITA-Glu** FGNs.

2. Experimental

2.1. Materials

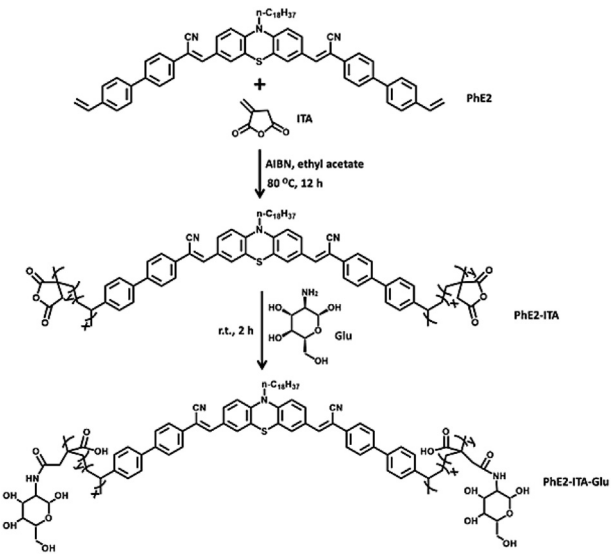
Phenothiazine, 1-bromooctadecane, 1,2-dichloroethane, phosphoryl chloride, 2-(4- bromophenyl) acetonitrile, 4-vinylphenylboronic acid, Aliquat 336, tetrakis(triphenylphosphine) palladium(0), tetrabutylammonium hydroxide, N,N- dimethylacetamide (DMAc), azobisisobutyronitrile purchased from Alfa Aesar were used as received. Itaconic anhydride and glucosamine hydrochloride were purchased from J&K Scientific Ltd. and used as received. All of other agents were obtained from commercial sources and used as received. Ultra-pure water was used through the whole experiments.

2.2. Instrumentals

The chemical structures were characterized by ^{1}H NMR spectroscopy and FTIR spectroscopy with a JEOL 400 MHz spectrometer and a Perkin–Elmer Spectrum 100 spectrometer (Waltham, MA, USA) in transmission mode, respectively. Gel permeation chromatography (GPC) analyses were performed on a Shimadzu LC-20AD pump system. The X-ray photoelectron spectra (XPS) were studied by a VGESCALAB 220-IXL spectrometer using an Al Kα X-ray source (1486.6 eV). The optical properties were characterized with UV/Vis/NIR Perkin–Elmer lambda750 spectrometer and PE LS-55 spectrometer (Waltham, MA, USA). The morphology and size distribution of **PhE2-ITA-Glu** FGNs were characterized by transmission electron microscopy (TEM) images using a JEM-1200EX microscope and a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY). The detailed conditions were the same with those described in our previous reports.

2.3. Preparation of PhE2-ITA-Glu FGNs

The AIE monomer **PhE2** was synthesized according to the literature methods.[49] In order to prepare **PhE2-ITA-Glu**, firstly **PhE2** was cross-linked with **ITA** through free radical polymerization, then ring-opening reaction of **ITA** by **Glu** was facilely conducted at room temperature under air atmosphere, affording the amphiphilic polymer (Scheme 2). To be specific, **PhE2** (36.4 mg, 0.040 mmol), **ITA** (45 mg, 0.40 mmol), and AIBN (5.0 mg) were dispersed in 6 mL ethyl acetate first. The above mixture was put into a schlenk tube and purged with nitrogen flow for about 30 min. Then the tube was set into an oil bath of 80 °C for 12 h under stirring. **Glu** (86 mg, 0.40 mmol) was dispersed in 6 mL of methanol, to which five drops of triethylamine were added, and stirred at 60 °C for 12 h. Then **Glu** solution in methanol was added to the above tube, being stirred for another 2 h at room temperature. Afterwards, the reaction was ended and the solution was dialyzed using 7000 Da Mw cutoff dialysis membranes against tap water and ethanol for 24 h and 6 h, respectively. Finally, the solution in dialysis bag was freeze-dried to afford the product (Scheme 2).



Scheme 2. Synthetic route of **PhE2-ITA-Glu**.

2.4. Cell culture

Human lung adenocarcinoma epithelial (A549) cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO_2. Culture medium was changed every two days in order to keep the exponential growth of the cells.

2.5. Cytotoxicity of PhE2-ITA-Glu FGNs

The effects of **PhE2-ITA-Glu** FGNs to A549 cells can be examined by checking the changes of cell morphology and number. Firstly, cells were seeded in 6-well microplates with a density of 7×10^4 cells mL^{-1} in 2 mL culture media. After cell attachment, different concentrations of **PhE2-ITA-Glu** FGNs prepared in culture media

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