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Stable biocompatible cross-linked fluorescent polymeric nanoparticles based on AIE dye and itaconic anhydride

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ABSTRACT

Self-assembly of polymeric materials to form nanoparticles is a particularly promising strategy for various biomedical applications, however, these self-assembling systems often encounter the critical micelle concentration (CMC) issue, as the nanoparticles is usually unstable at low concentration. Therefore, stable cross-linked fluorescent polymeric nanoparticles (FPNs) were covalently constructed from an aggregation induced emission (AIE) dye, itaconic anhydride, poly(ethylene glycol) monomethyl ether methacylate and polyethylenimine. These obtained PhE-ITA-20%(80%) FPNs were fully characterized by a series of techniques including ^{1}H NMR spectra, UV–vis absorption spectra, fluorescence spectra, FT-IR spectra, transmission electron microscopy, gel permeation chromatography, and dynamic light scattering. Such FPNs emitted intense fluorescence due to the introduction of aggregation induced emission dye. More importantly, the FPNs were found extremely stable in physiological solution even below the CMC owing to their cross-linked architectures. Biocompatibility evaluation and cell uptake behavior of the FPNs were further investigated to explore their potential biomedical applications, the demonstrated excellent biocompatibility made them promising for cell imaging.

1. Introduction

Cellular fluorescence imaging has attracted much attention as a versatile visualization way for medical diagnosis, drug development, and clinical study [1,2]. Various fluorescent bioprobes have been reported and extensively studied for bioimaging applications over the past decades, among them are green fluorescent protein, semiconductor quantum dots, carbon dots, and organic dye based fluorescent nanoparticles [3–6]. However, green fluorescent protein often suffers from small stokes shifts, poor photostability, and tedious transfection process [7]. While semiconductor quantum dots are usually easy to aggregate in cellular environments leading to high cytotoxicity owing to the containing heavy metal ions [8]. Carbon dots show weak luminescence and non-functionalized hydrophobic feature, which limit their biomedical application [9,10]. For most organic dyes, hydrophobic planar structures will induce strong intermolecular π–π interactions, resulting in fluorescence quenching and photobleaching when aggregated in aqueous solution [11,12]. To conquer the aggregating fluorescence quenching problem, another type of unique organic dyes emerges, firstly named aggregation-induced emission (AIE) dyes by Tang et al. [13]. The subsequent development of AIE dyes have given birth to a wide spectrum of molecular architectures, such as siloles [14], cyano-substituted diarylethylene [15], distyrylanthracene [16], tetraphenylethene [17], triphenylethene [18], and so on. Meanwhile, progressively investigation of these AIE materials for potential chemosensors and bioimaging applications has been proceeding [19].

Recently, various strategies for fabricating AIE dye based fluorescent nanoparticles have been developed. Prasad et al. [20] reported organically modified silica nanoparticles encapsulating a varying amount of AIE dye (BDSA), which provided a promising pathway to achieve a significant breakthrough in developing two-photon fluorescent probes for cell imaging. Jen et al. [21] presented a simple and novel method of utilizing AIE molecules as fluorescent probes for bioimaging *via* employing amphiphilic block copolymers to form polymeric micelles and function as nanocarriers to disperse hydrophobic AIE dyes. Tang and Liu et al. [22] utilized bovine serum albumin as the polymer matrix to encapsulate an AIE red fluorogen, which showed high brightness and low cytotoxicity for *in vitro* and *in vivo* far-red/near-infrared bioimaging. Wei's group developed surfactant modification of AIE dye with commercialized non-ionic surfactant Pluronic F127 to afford biocompatible

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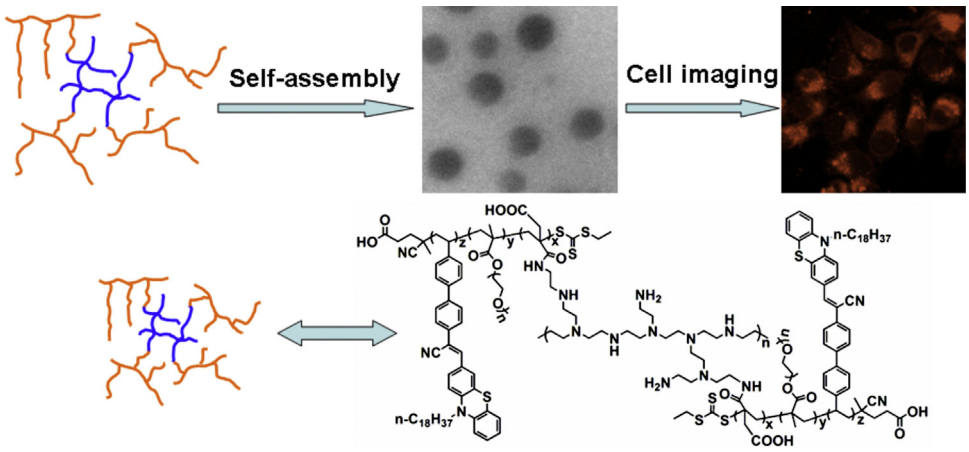

Scheme 1. Self-assembly of **PhE-ITA-20%**(**80%**) to form FPNs and their cell imaging applications.

fluorescent nanoparticles with good water solubility and excellent biocompatibility for cell imaging [23,24]. Although the above encapsulating strategies show good biological imaging capability, these non-covalent tactics will lead to dye leakage from the matrix, resulting in cytotoxicity when applied in bioimaging [25]. For biological applications, robust water-soluble AIE based nanoparticles are required, and the covalent strategy is a promising one. Therefore, AIE molecules grafted amphiphilic biopolymer chitosan was introduced by Tang et al. [26] to construct covalent AIE dye based amphiphilic polymer for cell imaging. Moreover, Wei' group has reported several covalent strategies to fabricate AIE dye based polymer, including Schiff base condensation [27], emulsion polymerization [28], reversible addition fragmentation chain transfer (RAFT) polymerization [29], and anhydride ring-opening polymerization [30]. Despite many impressive advances in fabricating AIE based macromolecules, more versatile and robust strategies are still highly demanded. As many previously reported AIE based fluorescent polymers are linear polymers, which are not stable on dilute solution below the CMC, and will limit their real biomedical application [31]. In this case, cross-linked polymeric nanoparticles have been expected more stable than those non-crosslinked ones. However, the reported example of cross-linked polymeric nanoparticles for cell imaging is still rare, and the related construction methodology is also limited [32–34]. Thus, development of robust synthetic routes to prepare novel stable cross-linked fluorescent polymeric nanoparticles (FPNs) is of great scientific interest.

Biobased materials have attracted much attention due to the ever-growing environmental problems, such as, consuming fossil fuel resources increases the net amount of carbon dioxide in the atmosphere and affects the global warming eventually [35]. Thus, it is considered very important to do research on polymer synthesis using renewable resources as starting materials. Itaconic acid is produced in a large scale by fermentation process and itaconic anhydride (ITA) is also regarded as one of key platform chemicals derived from biomass, which belongs to a renewable resource [36].

In this work, stable cross-linked copolymers **PhE-ITA-20%**(**80%**) were covalently constructed from renewable monomer ITA, an AIE dye (**PhE**), poly(ethylene glycol) monomethyl ether methacylate (PEGMMA), and polyethylenimine. The obtained amphiphilic cross-linked copolymers were prone to self-assembly into stable FPNs and could be highly dispersed in physiological solution (Scheme 1). Then, a series of techniques including ^{1}H NMR spectra, UV–vis absorption spectra, fluorescence spectra, FT-IR spectra, transmission electron microscopy, gel permeation chromatography, and dynamic light scattering were conducted to thoroughly characterize these FPNs. Meanwhile, the biocompatibility and cell uptake behavior of **PhE-ITA-20%**(**80%**) FPNs were studied to evaluate their potential applications for cell imaging.

2. Experimental procedure

2.1. Materials and characterization

Phosphoryl chloride, N,N-dimethylformamide, 2-(4-bromophenyl)acetonitrile, tetrabutyl ammonium bromide, tetrakis(triphenylphosphine) palladium(0), Aliquat 336, 1,2-dichloroethane, 4-vinylphenylboronic acid, terabutyl ammonium hydroxide, itaconic anhydride, poly(ethylene glycol) monomethyl ether methacylate (M_n = 950), polyethylenimine (M_n = 600) were purchased from J&K Scientific Ltd. and used as received. All other agents and solvents were purchased from commercial sources and used directly without further purification. Ultra-pure water was used in the experiments.

^{1}H NMR spectra were measured on a Mercury-Plus 300 MHz spectrometer [d_6-DMSO as solvent and tetramethylsilane (TMS) as the internal standard]. The FT-IR spectra were obtained in a transmission mode on a Shimadzu Spectrum 8400 spectrometer (Japan). Typically, 8 scans at a resolution of 1 cm^{-1} were accumulated to obtain one spectrum. UV–vis absorption spectra were recorded on UV/Vis/NIR 2600 spectrometer (Shimadzu, Japan) using quartz cuvettes of 1 cm path length. Fluorescence spectra were measured on an F-4600 spectrometer with a slit width of 3 nm for both excitation and emission. Transmission electron microscopy (TEM) images were recorded on a HT7700 microscope (Hitachi, Japan) operated at 100 kV, the TEM specimens were made by placing a drop of the nanoparticles suspension on a carbon-coated copper grid. The size distributions and zeta potential measurements of **PhE-ITA-20%**(**80%**) FPNs in phosphate buffer solution (PBS), in cell culture medium, and in serum were determined using a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY). Gel permeation chromatography (GPC) analyses of polymers were performed using DMF as the eluent. The GPC system was a Shimadzu LC-20AD pump system comprising of an auto injector, a MZ-Gel SDplus 10.0 mm guard column (50 × 8.0 mm, 10^2 Å) followed by a MZ-Gel SDplus 5.0 μm bead-size columns (50–10^6 Å, linear) and a Shimadzu RID-10A refractive index detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10^6 g mol^{-1}.