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Facile preparation and biological imaging of luminescent polymeric nanoprobes with aggregation-induced emission characteristics through Michael addition reaction

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ABSTRACT

Water dispersion aggregation-induced emission (AIE) dyes based nanomaterials have recently attracted increasing attention in the biomedical fields because of their unique optical properties, outstanding performance as imaging and therapeutic agents. The methods to conjugate hydrophilic polymers with AIE dyes to solve the hydrophobic nature of AIE dyes and makeS them widely used in biomedicine, which have been extensively explored and paid great effort previously. Although great advance has been made in the fabrication and biomedical applications of AIE-active polymeric nanoprobes, facile and efficient strategies for fabrication of biodegradable AIE-active nanoprobes are still high desirable. In this work, amphiphilic biodegradable fluorescent organic nanoparticles (PLL-TPE-O-E FONs) have been fabricated for the first time by conjugation of AIE dye tetraphenylethene acrylate (TPE-O-E) with Poly-L-Lysine (PLL) through a facile one-step Michael addition reaction, which was carried out under rather mild conditions, included air atmosphere, near room temperature and absent of metal catalysts or hazardous reagents. Due to the unique AIE properties, these amphiphilic copolymers tend to self-assemble into high luminescent water dispersible nanoparticles with size range from 400 to 600 nm. Laser scanning microscope and cytotoxicity results revealed that PLL-TPE-O-E FONs can be internalized into cytoplasm with negative cytotoxicity, which implied that PLL-TPE-O-E FONs are promising for biological applications.

1. Introduction

Over the past few decades, fluorescent nanomaterials have been extensively explored for biomedical applications because of their outstanding optical properties as compared with the small organic dyes [1,2]. Typically, fluorescent nanomaterials could be mainly classified as fluorescent organic nanoparticles (FONs) and fluorescent inorganic nanoparticles (FINs) [3–6]. These fluorescent nanoparticles have been widely used for biomedical applications because of their high sensitivity, rapid response, non-destructive character and signal stability that can visualize the morphological details of cells [7,8]. Promising fluorescent nanoparticles for biomedical applications should possess following features, which included desirable optical properties, high water dispersibility, biodegradable potential and good biocompatibility [9–18]. As compared with FINs, FONs showed obvious advantages for bioimaging applications for their designability, biodegradability and biocompatibility. However, it is well known that the fluorescence of FONs based on the conventional organic dyes will largely quenched due to the aggregation-caused quenching (ACQ) effect, which will occur in high concentrations or in solid state [19–22]. This fatal flaw makes us challengeable to prepare ultrabright FONs using the conventional organic dyes. Since Tang et al. discovered and proposed the aggregation-induced emission (AIE) in 2001 [20], more and more attention has been attracted by this abnormal fluorescence phenomenon. Opposite to the ACQ effect, AIE active dyes emitted much stronger fluorescent intensity in their aggregate state due to the restriction of intramolecular rotations (RIR) and prohibition of energy dissipation via non-irradiative channels [19]. To date, a large number of new AIE luminogens containing silole,

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tetraphenlyethene (TPE), triphenylamine (TPA), 9,10-distyrylanthrancene (DSA), 1,4-distyrylbenzene (DSB) and their derivatives have been designed and explored for different applications [23–29]. However, the inherent hydrophobic features of AIE fluorogens have limited their application in physiological solution, which is of great challenges to modify these AIE-active fluorogens with stable and excellent water dispersibility. Since amphiphilic polymers are generally intended to self-assemble into vesicle/micelle-like uniform structures in aqueous solutions [23,30–33]. A variety of FONs had been reported by Tang et al. and Zhang et al. based on AIE-active dyes [28,34–44], which showed good water dispersibility and biocompatibility.

Poly-L-lysine (PLL) has plentiful active amino groups, which can covalently connect with drugs or be modified for further application. Besides, PLL is also an ideal gene delivery system because of the plentiful positively charged amino groups. And most importantly, PLL is a water-soluble, low-cost and commercial available polymer, which can be easily degraded into digestible lysine. Therefore, the PLL based materials can also avoid long-time retention in tissue after they were accumulated by tissues through enhanced permeability and retention effect (EPR) [45]. PLL-functionalized nanomaterials have been widely used in cell labeling, DNA electrochemical sensors, and gene or drug delivery [46,47]. PLL is biodegradable, nontoxic, non-immunogenic and non-inflammatory, which makes it an ideal carrier for systemic drug delivery applications. However, the fabrication of AIE-active polymeric nanoprobes with good water dispersibility, biocompatibility and biodegradable has rarely reported [48,49]. For example, Zheng et al. have described the conjugation of AIE-active dye with chitosan through the covalent reaction between isocyanate group of AIE dye and the amino group of chitosan [49]. Park et al. have also developed a covalent method for fabrication of aggregation induced emission enhancement nanoprobes through conjugation between the carboxyl group of AIE dye and the amino groups of water-soluble glycol chitosan [48]. The Michael addition reaction between amino group and ene bond is a simple, fast and efficient method for synthesis of many useful materials (e.g. polyamidoamine dendrimers) in mild reaction conditions [50,51]. It can be occurred under near room temperature, absent of metal catalysts and air atmosphere. Therefore, it is very useful for fabrication of biomaterials but maintaining their biological active and the luminescent properties. However, to the best of our knowledge, the fabrication of AIE-active luminescent nanoparticles using Michael addition reaction has not been reported thus far.

In this contribution, we reported a rapid and simple method to prepare water dispersible FONs based on AIE-active dyes with biodegradable PLL as a linker through a facile Michael addition reaction (Scheme 1). The PLL-functionalized AIE active materials (PLL-TPE-O-E FONs) were high water dispersibility, biodegradable and nontoxic. With plentiful amino groups, these AIE active nanoparticles can be further modified with bioactive molecules, targeting agents and other imaging agents, and present great potential application in future. The AIE dyes as well as the final PLL-TPE-O-E FONs were characterized by a number of techniques to determine their structure and properties in detail. As compared with other fabrication methods, the Michael addition reaction can occur under rather mild conditions (e. g. near room temperature 35 °C, air atmosphere, absent of metal catalysts and in the present of water, neutral pH). Therefore, the Michael addition reaction is specific useful for conjugation of AIE dyes with many other biomacromolecules such as proteins, peptides and synthetic molecules with amino groups but can well keep their structure and bioactivity.

2. Materials and methods

2.1. Materials and characterization

Bromotriphenylethylene, tetrakis (triphenylphosphine) palladium, etrabutyl ammonium bromide (TBAB), 4-carboxybenzeneboronic acid and acryloyl chlorid (99%) purchased from Aladdin (Shanghai, China) were used as received. Anhydrous tetrahydrofuran (THF, J&K Chemical), ethyl acetate (J&K Chemical) and triethylamine (Et3N, J&K Chemical, 99.5%) were used without further purification. ^{1}H nuclear magnetic resonance (NMR) spectra were performed on Bruker Avance-400 spectrometer with D_2O or $CDCl_3$ as solvent and tetramethylsilane (TMS) as reference. The Fourier transform infrared (FT-IR) spectra were carried out by using a Nicolet 380 Fourier transform spectrometer with a resolution of 2 cm^{-1}. PerkinElmer LAMBDA 35 UV/vis system was used to measure UV–vis absorption spectrum. Transmission electron microscopy (TEM) images were recorded on a Hitachi 7650B microscope operated at 80 kV, and the specimens were made by dropping a drop nanoparticle suspension on a carbon-coated copper grid. Fluorescence spectra (FL) were tested through a PELS-55 spectrometer with a slit width of 3 nm for the emission of PLL-TPE-O-E FONs in water. The quantum yield of PLL-TPE-O-E FONs in aqueous solution was determined using quinine sulfate as reference dye. The quinine sulfate was dispersed in 0.05 mol L^{-1} sulfuric acid aqueous solutions (the concentration of quinine sulfate is 1×10^{-3} mol L^{-1}). The adsorption values of PLL-TPE-O-E FONs and quinine sulfate was determined using UV–vis spectrometer. The fluorescence spectra of PLL-TPE-O-E FONs and quinine sulfate excited at 360 nm were recorded on PELS-55 spectrometer. The integrated areas based on their fluorescence spectra (wavelength range 380–580 nm) were calculated. The relative QY of PLL-TPE-O-E FONs was calculated using the formula as described in our previous report [14]. Zeta-potential and size distribution of PLL-TPE-O-E FONs in water were measured using a Zeta Plus particle size analyzer (Zeta Plus, Brookhaven Instruments, Holtsville, NY).

2.2. Synthesis of TPE-O-E

TPE-O-E was performed through two steps. In the first step, the synthetic route of TPE-OH was carried out according to Zhang et al. [52,53] with little changes as following: Bromotriphenylethylene (1.675 g, 10 mmol) and 4-Carboxybenzeneboronic acid (2.25 g, 15 mmol) were dissolved in the 60 mL tetrahydrofuran (THF), then, 0.32 g TBAB and 2 M potassium carbonate aqueous solution (18 mL) were mixed with the solution. After the mixture was stirred at 55 °C under nitrogen for about 0.5 h, Pd $(PPh_3)_4$ (0.010 g, 8.70×10^{-3} mmol) was added in the mixture and then heated to 90 °C for 24 h. The process was monitored by thin layer chromatographic (TLC). After extracted from water and ethyl acetate, the organic layer was dried over anhydrous sodium sulfate (Na_2SO_4). After evaporated under vacuum, the clued product was purified on silica gel with a mixture of *n*-hexane and CH_2Cl_2 (volume ratio 2:1) to product pure compound TPE-OH. The product was confirmed by ^{1}H NMR ($CDCl_3$ as solvent) and FT-IR.

In the second step, 417.6 mg TPE-OH (1.2 mmol) was reacted with 350 mg acryloyl chloride (3.6 mmol) at a 1:3 molar ratio in 10 mL absolute THF, and then triethylamine (1 mL) was added dropwise. The reaction was stirred in ice-bath under nitrogen until TPE-OH completely reacted (the process was about 6 h and monitored by TLC). After filtered under vacuum, the solution was extracted with saturated sodium bicarbonate three times to remove unreacted chemical agents. The organic phase was