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Fabrication and biological applications of luminescent polyamidoamine dendrimers with aggregation-induced emission feature

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ABSTRACT

The luminescent polymeric nanoparticles (LPNs) with aggregation-induced emission (AIE) properties have emerged as one of the most promising nanoprobes for their unique optical properties. The typical optical feature of AIE-active LPNs should be their obviously enhanced luminescence in aggregation or solid state, which can effectively overcome aggregation caused quenching effects of conventional organic dyes. In this study, we reported for the first time that highly emissive LPNs (named as TPE-E-PAMAM) can be simply fabricated via direct conjugation of tetraphenylethene derivative (TPE-E) with polyamidoamine (PAMAM) dendrimers, which relied on a one-step Michael addition reaction between ene group of TPE-E and amino group of PAMAM dendrimers under rather mild experimental conditions. Because of the strong intermolecular interaction of TPE-E PAMAM copolymers, they can form compact spheres in water and exhibit strong fluorescence in aqueous solution. TPE-E PAMAM LPNs possess high water dispersity, uniform morphology and desirable biocompatibility for biological imaging. More importantly, with large numbers of amino groups on the shell and valid space in the core, PAMAM-TPE-E LPNs have great potential for targeted gene delivery. Taken together, we described a facile one-step covalent strategy for developing AIE-active amphipathic dendrimers, which showed great potential for biomedicine applications.

1. Introduction

Fluorescence nanostructures have been widely used in biological studies due to its high sensitivity, diversity and non-destructive features, which can real-time, visualize the physiological alteration and monitor biological processes in living organisms. Numerous of fluorescence nanoparticles with different compositions and optical properties have been developed and widely applied in biological imaging, molecular diagnosis and intracellular gene/drug delivery [1-7]. Especially the luminescent nanoparticles polymeric (LPNs) with strong fluorescent intensity, low cytotoxicity and biodegradable potential have attracted the attention in recent years [8-13]. Self-assembly of amphiphilic luminescent copolymers is the commonly adopted method for fabrication of these LPNs. However, the LPNs based on conventional organic dyes often suffer from the notorious aggregation-caused quenching effect (ACQ), which was caused by $\pi-\pi$ stacking of hydrophobic dyes during the self-assembly procedure [14-17]. In 2001, Tang and co-workers reported the abnormal fluorescence emission phenomenon that showed that the organic dye (1-methyl-1, 2, 3, 4, 5-pentaphenylsilole) could emit much strong luminescence in its solid state. This phenomenon was named as aggregation induced emission (AIE), which provided an elegant route to fabricate LPNs with strong luminescence due to the unique optical feature of AIE-active dyes [18]. With the mechanism that restriction of intramolecular motion (RIM) suppresses the nonradiative pathway and switches on the radiative channel, a large numbers of AIE-active dyes have been synthesized and explored for biosensing and imaging applications [19-28]. Several methods have been employed to modify AIE-active dyes with recognition elements and hydrophilic groups to endow them specific affinity and water dispersion. Liu's group has conjugated both hydrophilic linker and targeting ligand with TPE derivates for specific cell thiol imaging via the coupling reaction [29]. Zhang and other researchers have also developed a

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number of water dispersion AIE-active LPNs through non-covalent self-assembly, covalent linkage, emulsion polymerization and RAFT polymerization, formation of dynamic bonds and so on [30-34]. However, developing an effective and simple method to fabricate water dispersible AIE-active LPNs with large numbers of functional groups and specific molecular structure is still desirable.

Polyamidoamine (PAMAM) dendrimers, as highly branched nanostructured macromolecules with monodispersed mass, multifunctional surface and encapsulated core, have been keeping them in the spotlight [35-39]. Because of the unique tree-like branching architecture that originates from an initiator core as concentric, symmetrical monomer branching shells, PAMAM dendrimers have been widely used in various fields, such as drug delivery, catalysis supports, DNA or siRNA delivery and MRI contrast agents [22,40-52]. PAMAM dendrimers can form into spherical structures in solution through the electrostatic interaction of ammonium-carboxylate ion pairs. They can cross cell barriers by both paracellular and transcellular pathways. So, drugs can be attached to dendrimer terminal groups via covalent linker or encapsulated in void spacers through the electrostatic interaction. Meanwhile, targeting moieties can be designed to be conjugated to the surfaces, which can deliver drugs to targeted disease sites effectively. All these characteristics make PAMAM dendrimers ideal delivery vectors. Compared to linear polymers, dendrimers can form a tightly packed ball in water with lower viscosity. Unlike conventional micelles, dendrimers do not have a critical micelle concentration (CMC) because of the unimolecular nature [53]. So, the micelles still maintained thermodynamically stable when they are injected into the bloodstream and subjected to severe dilution, which can prevent the burst-release of entrapped drugs. Since both AIEgens and PAMAM dendrimers have been the focus of many research and have broaden future in biomedicine application, bringing them together can open a new breakthrough not only in imaging application but also in studying drug release mechanism. The small internal cavities can restrict the intramolecular rotation of AIE chromophores and boost fluorescence. Meanwhile, the numerous amino groups on the shell can make it soluble in physiological solution. However, only one study has demonstrated the fabrication of AIE-active PAMAM dendrimers through non-covalent interaction [25].

In this work, a facile one-step strategy was developed to fabricate AIEgens based PAMAM dendrimers. Tetraphenyl ethylene derivatives (TPE-E) with ene bond was conjugated with PAMAM dendrimers ($G = 3.0$) through Michael addition reaction, which can occur under rather mild experimental conditions such as air atmosphere, low temperature, absent of metal catalysts in benign solvents. The amphiphilic AIE-active nanoparticles (TPE-E-PAMAM LPNs) not only showed high water-solubility due to the presence of PAMAM dendrimers but also exhibited strong fluorescence for the AIE properties of TPE-E. The nanoparticles have been applied into HeLa cells and showed strong fluorescence and high cell uptake efficiency. With the unique characteristics of both PAMAM dendrimers and TPE-E, the TPE-E-PAMAM LPNs have the potential to be widely used in biomedicine in future.

2. Materials and methods

2.1. Materials

Bromotriphenylethylene, tetrakis palladiumbrt (triphenylphosphine), tetrabutyl ammonium bromide (TBAB), 4-Carboxybenzeneboronic acid, methyl alcohol, and acryloyl chloride (99%) purchased from Aladdin (Shanghai, China) were used as received. PAMAM dendrimers (MW: 6909 Da, Gn = 3.0) were pursed from chemical new material company of WeiHai Chengyuan. 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolim assays (MTS; Promega, USA), anhydrous tetrahydrofuran (THF, J&K Chemical), ethyl acetate (J&K Chemical) and triethylamine (Et3N, J&K Chemical, 99.5%) were used without further purification. Penicillin–streptomycin, fetal bovine serum (FBS) and trypsin-EDTA solution were purchased from Sigma-Aldrich.

2.2. Instrumentation

^{1}H NMR spectra were recorded on a Bruker Avance-400 spectrometer with D_2O or $CDCl_3$ as solvents and tetramethylsilane (TMS) as internal standard. Photoluminescence (PL) spectra were obtained on a PELS-55 spectrometer. The UV–vis absorption spectra were measured by a Perkin Elmer LAMBDA 35 UV/Vis system. The Fourier transform infrared (FT-IR) spectra were performed on a Nicolet 380 Fourier transform spectrometer with a resolution of 2 cm^{-1}. The size and morphology of samples was carried out using transmission electron microscopy (TEM) by drop of a drop particle ethanol suspension on a carbon-coated copper grid.

2.3. Synthesis of TPE-E

The schematic diagram illustrating the synthesis route was presented in Scheme 1. The reaction intermediates tetraphenyl ethylene-acrylate (TPE-E) was carried out according to the previously described method [54]. Briefly, bromotriphenylethylene (1.675 g, 10 mmol) and 4-carboxybenzene boronic acid (2.25 g, 15 mmol) were dissolved in the 60 mL THF, then, 0.32 g TBAB and 2 M potassium carbonate aqueous solution (18 mL) were added in 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, after purified, the product(TPE-O) was obtained. 417.6 mg TPE-O (1.2 mmol) and 350 mg acryloyl chloride (3.6 mmol) was mixed in 10 mL absolute THF, and then triethylamine (1 mL) was added dropwise. The reaction was stirred in ice-bath under nitrogen until TPE-O completely reacted. The product TPE-E was acquired after dried under vacuum.

2.4. Synthesis of TPE-E-PAMAM

Unlike oil-in-water (O/W) single emulsion solvent evaporation method, the TPE-E-PAMAM LPNs were synthesized by one-step Michael addition reaction through the reaction of ene bond and amino group. TPE-E (50 mg) was dissolved in 6 mL ethyl acetate and PAMAM (270 mg) was dissolved in 2 mL methyl alcohol, then the mixture was stirred at room temperature for 8 h (monitored by TLC until no TPE-E was rest). Then, the polymer solution was washed with water and extracted with ethyl acetate. After dried through anhydrous Na_2SO_4, organic solvents were removed on a rotary evaporator at 50 °C under vacuum to obtain pure TPE-E-PAMAM.

2.5. Cytotoxicity of TPE-E-PAMAM NPs and cellular imaging

The cytotoxicity of TPE-E-PAMAM NPs against HeLa cells was assessed by using the MTS assay. The cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, penicillin/streptomycin, and L-glutamine at 37 °C with 5% CO_2 with a density of 4×10^4 cells/well for 24 h. Then, the medium was replaced with fresh medium containing various concentrations of TPE-E-PAMAM LPNs (0, 10, 20, 40, 80, 120 μg/mL) and then were incubated for 8 and 24 h, respectively. MTS assay was performed as a standard of cytotoxicity of TPE-E-PAMAM by measuring absorbance at 570 nm. The experiment was performed in triplicate. Cell viability rate was calculated as percentage compared to the control cells: (absorbance of the treated wells)/(absorbance of the control wells) × 100%.

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