Colloids and Surfaces B: Biointerfaces 126 (2015) 273–279

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

One-pot preparation of cross-linked amphiphilic fluorescent polymer based on aggregation induced emission dyes

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ARTICLE INFO

Article history:
Received 23 November 2014
Received in revised form
11 December 2014
Accepted 15 December 2014
Available online 3 January 2015

Keywords:
Aggregation induced emission
Cross-linked
Amphiphilic fluorescent polymer
nanoparticles
Imaging

ABSTRACT

Facile one-pot preparation of cross-linked amphiphilic fluorescent polymer based on aggregation induced emission (AIE) dyes and 2-isocyanatoethyl methacrylate (IM) has been developed. This was carried out first by free radical polymerization between AIE monomer (PhE) and IM, and then polyethyleneimine (PEI) was introduced to obtain the cross-linked fluorescent polymer. The resulted cross-linked amphiphilic polymer was prone to self-assemble into stable nanoparticles in aqueous solution with surplus amino groups on the surface which made them highly water dispersible and can be further functionalized. The as-prepared fluorescent polymer nanoparticles (PhE-IM-PEI FPNs) were fully characterized by a series of techniques including ^{1}H NMR spectrum, X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy, transmission electron microscopy, dynamic light scattering, UV–vis absorption spectrum, and fluorescence spectra. Such FPNs demonstrated intense orange fluorescence with a high quantum yield of about 40%. Biocompatibility evaluation and cell uptake behavior of the nanoparticles were further investigated to explore their potential biomedical applications; the demonstrated excellent biocompatibility made them promising for cell imaging.

1. Introduction

Fluorescent polymeric nanoparticles (FPNs), which mean fluorophore-labeled polymeric systems, have attracted great interest for sensing, bioimaging and biomedical applications, due to their simple operation, fluorescent characteristics and prompt response [1–8]. Compared with their small-molecule counterparts, FPNs are much more promising for biological applications because of water dispersibility, their biocompatibility, and facile synthesis strategy with functional groups for modification, etc. [9–13]. Furthermore, the good permeability of the cell membrane or nucleus, which is closely related to many diseases, makes these nanoparticles a desirable system for drug delivery and cell imaging [14–17]. However, for most small-molecule fluorophores, hydrophobic planar structures will induce strong intermolecular π–π interactions, resulting in fluorescence quenching and photobleaching when aggregated in aqueous solution, which is known as aggregation-caused quenching (ACQ) [18–20]. FPNs prepared utilizing these fluorophores would also encounter the same problem, which would severely limit their real biomedical applications. In order to solve this ACQ problem, another type of unique organic dyes was developed, which can emit much stronger luminescence in their aggregation states. These organic dyes were first reported by Tang et al. in 2001 and were called aggregation induced emission (AIE) dyes [19,21]. More importantly, a lot of AIE fluorogens such as siloles, tetraphenylethene, triphenylethene, cyano-substituted diarylethene, and distyrylanthracene derivatives have already been synthesized and extensively investigated for chemosensors and bioimaging applications [22–28].

Recently, many kinds of AIE dyes based FPNs have been rapidly developed and have received much attention owing to their facile processability, good solubility, and high emission efficiency in the aggregated states, etc. [25,29–33]. By now, two major strategies for constructing AIE dye based FPNs have been developed including a physical method and a chemical method. The physical method is mainly to encapsulate the AIE dyes with biocompatible amphiphilic polymers to afford FPNs [34–36]; however, dye leakage or surface coating detachment is the main obstacle in this non-covalent system. On the other hand, the chemical method to prepare FPNs comprises Schiff-base reaction, reversible addition

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http://dx.doi.org/10.1016/j.colsurfb.2014.12.025
0927-7765/

fragmentation chain transfer (RAFT) polymerization, emulsion polymerization, ring-opening polymerization, and cross-linked polymerization [33,37–40]. Despite many impressive advances in fabricating AIE based macromolecules, more versatile and robust strategies are still highly demanded. As most of aforementioned AIE based fluorescent polymers are linear polymers, which are not stable in dilute solution below the critical micelle concentration, and will limit their real biomedical application [41,42]. In this case, cross-linked polymeric nanoparticles have been expected more stable than those non-crosslinked ones. However, the related construction methodology of cross-linked FPNs based on AIE dyes is still limited [29,43–45].

In this work, a facile strategy to prepare AIE dyes based cross-linked FPNs (PhE-IM-PEI FPNs) was developed by one-pot method for the first time. Firstly, free radical polymerization between AIE monomer (PhE) and 2-isocyanatoethyl methacrylate (IM) was conducted, and then polyethyleneimine (PEI) was introduced to obtain the cross-linked fluorescent polymer. Such a PhE-IM-PEI amphiphilic polymer was prone to self-assemble into nanoparticles with great stability because of the cross-linked structures and high water dispersibility due to the surplus amino groups covered on the surface. The fluorescence property, morphology, and stability of the FPNs were further characterized by fluorescence spectrum, transmission electron microscopy, and dynamic light scattering. Finally, the biocompatibility and cell uptake behavior of PhE-IM-PEI FPNs were determined to evaluate their potential cell imaging applications (Scheme 1).

2. Experimental

2.1. Materials and methods

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, 2-isocyanatoethyl methacrylate, and polyethyleneimine 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.

Gel permeation chromatography (GPC) analyses 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 mm × 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}.UV–vis absorption spectra were recorded on UV/Vis/NIR Perkin-Elmer lambda750 spectrometer (Waltham, MA, USA) using quartz cuvettes of 1 cm path length. Fluorescence spectra were measured on a PE LS-55 spectrometer with a slit width of 3 nm for both excitation and emission. The FTIR spectra were obtained in a transmission mode on a Perkin-Elmer Spectrum 100 spectrometer (Waltham, MA, USA). Typically, 8 scans at a resolution of 1 cm^{-1} were accumulated to obtain one spectrum. ^{1}H NMR spectra were measured on a JEOL 400 MHz spectrometer [D_2O, $CDCl_3$ or d_6-DMSO as solvent and tetramethylsilane (TMS) as the internal standard]. The X-ray photoelectron spectra (XPS) were performed on a VGESCALAB 220-IXL spectrometer using an Al Kα X-ray source (1486.6 eV). The energy scale was internally calibrated by referencing to the binding energy (Eb) of the C1s peak of a carbon contaminant at 284.6 eV. Transmission electron microscopy (TEM) images were recorded on a JEM-1200EX microscope 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 distribution of PhE-IM-PEI FPNs in water and phosphate buffer solution (PBS) was determined using a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY).

2.2. Preparation of PhE-IM-PEI FPNs

The AIE monomer PhE was prepared according to the literature methods [43,45]. For synthesis of PhE-IM-PEI FPNs, PhE (34 mg, 0.050 mmol), IM (78 mg, 0.50 mmol), AIBN (5.0 mg), and ethyl acetate (6 mL) were introduced in schlenk tube and purged by nitrogen flow for 30 min. The above mixture was put into an oil bath maintained at 80 °C for 12 h. PEI (300 mg, 0.50 mmol) was added into the above mixture and stirred for another 2 h at room temperature. Afterwards, the reaction was stopped and dialyzed against tap water for 24 h and ethanol for 6 h using 7000 Da Mw cutoff dialysis membranes. Finally, this solution in the dialysis bag was freeze–dried to obtain the product (Scheme 2).

2.3. Cytotoxicity of PhE-IM-PEI FPNs

Cell morphology was used to examine the effects of PhE-IM-PEI FPNs to A549 cells. Briefly, cells were seeded in 6-well microplates at a density of 1×10^5 cells mL^{-1} in 2 mL of respective media containing 10% fetal bovine serum (FBS). After cell attachment, plates were washed with PBS and cells were treated with complete cell culture medium, or different concentrations of PhE-IM-PEI FPNs prepared in 10% FBS containing media for 24 h. Then all samples were washed with PBS three times to remove the uninternalized nanoparticles. The morphology of cells was observed by using an optical microscopy (Leica, Germany), the overall magnification was ×10.

The cell viability of PhE-IM-PEI FPNs on A549 cells was evaluated by cell counting kit-8 (CCK-8) assay based on our previous reports. Briefly, cells were seeded in 96-well microplates at a density of 5×10^4 cells mL^{-1} in 160 μL of respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 10, 20, 40, 80, 120 μg mL^{-1} PhE-IM-PEI FPNs for 8 and 24 h. Then nanoparticles were removed and cells were washed with PBS three times. 10 μL of CCK-8 dye and 100 μL of Dulbecco's modified Eagle's medium (DMEM) cell culture medium were added to each well and incubated for 2 h at 37 °C. Plates were then analyzed with a microplate reader (VictorШ, Perkin-Elmer). Measurements of formazan dye absorbance were carried out at 450 nm, with the reference wavelength at 620 nm. The values were proportional to the number of live cells. The percent reduction of CCK-8 dye was compared to controls (cells not exposure to PhE-IM-PEI FPNs), which represented 100% CCK-8 reduction. Three replicate wells were used per microplate, and the experiment was repeated three times. Cell survival was expressed as absorbance relative to that of untreated controls. Results are presented as mean ± standard deviation (SD).

2.4. Confocal microscopic imaging of cells using PhE-IM-PEI FPNs

A549 cells were cultured in DMEM supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} of streptomycin. Cell culture was maintained at 37 °C in a humidified condition of 95% air and 5% CO_2 in culture medium. Culture medium was changed every three days for maintaining the exponential growth of the cells. On the day prior to treatment, cells were seeded in a glass bottom dish with a density of 1×10^5 cells per dish. On the day of treatment, the cells were incubated with PhE-IM-PEI FPNs at a final concentration of 20 μg mL^{-1} for 3 h at 37 °C. Afterward, the cells were washed three times with PBS to remove the PhE-IM-PEI FPNs and then fixed with 4% paraformaldehyde for