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Ultrafast microwave-assisted multicomponent tandem polymerization for rapid fabrication of AIE-active fluorescent polymeric nanoparticles and their potential utilization for biological imaging

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ABSTRACT

The fabrication and biomedical applications of fluorescent polymeric nanoparticles (FPNs) with aggregation-induced emission (AIE) feature has attracted the most intensive research interest since the first discovery of AIE phenomenon by Tang' group. Although great attention has been devoted to preparation of AIE-active FPNs, an efficient, facile and versatile strategy is still highly desirable to advance their biomedical applications. In this work, a one-pot microwave-assisted multicomponent tandem polymerization was proposed to fabricate AIE-active FPNs based on a microwave-assisted Kabachnik–Fields (KF) reaction, which involves the conjugation of aldehyde group containing polyethylene glycol (CHO-PEG-CHO) and amino-group terminating AIE dye (H_2N-PhE-NH_2) using diethyl phosphate as the lock. The KF reaction can occur under rather facile and mild experimental conditions (e.g. absent of catalyst and solvents, air atmosphere) with the assistance of microwave irradiation in 5 min. The resultant (PEG-DP-PhE) copolymers would self-assemble into FPNs that showed high water dispersibility and enhanced fluorescence intensity. The desirable cytocompatibility and cell uptake efficiency of PEG-DP-PhE FPNs endow their great potential for biomedical applications. Considering the convenience and effectiveness, the method should be promising for fabrication of many AIE-active functional materials with great application potential.

1. Introduction

Fluorescence imaging is an emerging optical imaging technology in recent years [1,2]. It has been extensively explored for biological sensors and theranostics because of its high sensitivity, high spatial resolution and suitable for multi parameters measurement [3,4]. The applications of fluorescent nanoparticles for biological imaging have also made significant progress over the past few decades [5]. However, many of them have their own disadvantages that limit their further applications in the biomedical fields. For example, fluorescent proteins could be regarded as the water soluble and biocompatible fluorescent macromolecules at nanoscale range, while they are obtained through cumbersome transfection and purification processes and are therefore rather expensive [6]. On the other hand, fluorescent inorganic materials such as semiconductor quantum dots, metal clusters and carbon quantum dots are poor degradable and toxicity to living organisms owing to their heavy metal compositions and long term accumulation [7–12]. More importantly, the fluorescent properties of most fluorescent inorganic materials are difficult to be fine tuned through chemical structure design [13,14]. The fluorescent polymeric nanoparticles (FPNs) that composited with hydrophobic segments (organic dyes) and other hydrophilic components seem to be the most promising candidates as the fluorescent probes. However, the fluorescence intensity of these FPNs based on conventional organic dyes will be largely decreased owing to the notorious aggregation caused quenching (ACQ) effect from π-π stacking [15]. Therefore, the searching for novel organic dyes that could overcome the ACQ effect of conventional organic dyes is crucial for the fabrication of FPNs with desirable optical properties [16,17].

Since the first report of aggregation-induced emission (AIE)

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phenomenon in 2001 by Tang et al., this abnormal fluorescence phenomenon has rapidly attracted increasing research interest and has been extensively explored for various applications by Tang et al. [18–20]. Especially, the AIE-active functional materials have been widely for biomedical applications through the incorporation of AIE-active molecules into amphiphilic copolymers, which could self-assemble into AIE-active FPNs with desirable optical properties [21]. So far, there have been many reports on the construction of AIE-active FPNs have been developed by Wei et al. [22–24]. For example, we have previously demonstrated that the AIE-active FPNs could be facilely obtained through the non-covalent encapsulation using the commercially available surfactant F127 [25]. However, these methods are mainly relied on the hydrophobic interaction between AIE-active dyes and hydrophobic segments of amphiphilies. These AIE-active FPNs may not be stable in diluted solution when the concentration is lower than the critical micelle concentrations (CMC) of these amphiphilies. Many other constructed by covalent methods such as Schiff base reaction, free radical polymerization, controlled living radical polymerization as well as ring-opening reaction have also been developed by Zhang and Wei et al. [26–31]. However, these methods generally need tedious experimental procedures, harsh reaction conditions and long reaction time.

The multicomponent reactions (MCRs) were favored by organic synthetic chemists due to their simple operation of one-pot method, atom economy, mild conditions and low reaction costs and so on [32,33]. In recent years, the concept of MCRs has been introduced into the field of polymer chemistry. Therefore, MCRs came into being and have been extensively adopted in the synthesis of multifunctional polymers [34]. MCRs refer to introduce polymerizable elements in the MCRs, and functional polymers with clear structure and specific functional structure were facilely synthesized by the strategy of combining the functional monomers with controlled polymerization. Compared with the traditional controlled polymerization methods, such as atom transfer radical polymerization (ATRP) [35] and reversible addition-fragmentation chain transfer radical polymerization (RAFT) [36], MCRs not only do not need the participation of substances that may be harmful to the organism, such as catalysts and initiators, but also do not need the anaerobic conditions to obtain the final products with high efficiency. Therefore, using MCR strategy simplifies the experimental operation, reduces the cost. Meanwhile, the reaction conditions are more moderate, and the synthesis path is more green and economical. The Kabachnik–Fields (KF) multicomponent polymerization has been developed for the synthesis of high molecular weight polymers recently [37,38]. Take advantage of those merits mentioned above, we have proposed a novel microwave-assisted tandem polymerization method based on a Kabachnik–Fields (KF) reaction, which could greatly reduce the reaction time with the assistance of microwave irradiation [39]. On the other hand, the one-pot operation and solvent-free reaction conditions make this strategy more prominent.

In this contribution, a solvent-free and catalyst-free microwave-assisted multicomponent tandem polymerization (MCP) was developed to construct AIE-active FPNs. An AIE-active dye with two amino groups (Scheme S1) that named as H2N-PhE-NH2 was conjugated with the dialdehyde-terminated polyethylene glycol (named as CHO-PEG-CHO) through the KF reaction (Scheme 1). Due to the hydrophobicity of H_2N-PhE-NH_2 and hydrophilicity of PEG, the final conjugations (PEG-DP-PhE) are expected to be amphiphilic. The successful fabrication of these amphiphilic copolymers through the KF reaction based tandem polymerization was confirmed through a number of characterization techniques. The water dispersibility, optical properties as well as compatibility were also investigated to evaluate their potential biomedical applications. We demonstrated that the amphiphilic copolymers could be facilely obtained through the microwave-assisted MCP and the resultant fluorescent assemblies displayed good physicochemical properties and desirable biological imaging performance. Moreover, the microwave-assisted MCP should be a very promising method for fabrication of AIE-active FPNs for their high efficiency, short reaction time, mild experimental reaction conditions and substrate adaptability.

2. Experimental sections

2.1. Materials and characterization

N,*N′*-Dicyclohexylcarbodiimide (DCC), 4-formylbenzoic acid (CBA), polyethylene glycol (PEG, *M*w: 1000 Da), 4-dimethylaminopyridine (DMAP), diethyl phosphite and tetrahydrofuran (THF) offered from Aladdin (Shanghai, China) were all used as received. All other commercially available reagents were used without purification. The AIE dye NH_2-PhE-NH_2 was synthesized and characterized in our previous work [40]. The ^1H and ^{31}P NMR spectra were carried out on a Bruker Avance-400 spectrometer [D_2O and d_6-DMSO as the solvents and tetramethylsilane (TMS) as a reference]. Transmission electron microscopy (TEM) images were recorded on a Hitachi 7650B microscope operated at 80 kV. The FT-IR spectra were obtained in a reflection mode on a Nicolet5700 (Thermo Nicolet corporation) using KBr pellets. The UV–visible absorption spectra of nanoparticles in water solution were obtained on a Perkin Elmer LAMBDA 35 UV/Vis system using quartz cuvettes of 1 cm path length. The fluorescence spectra were measured by using a Fluorescence spectrophotometer (FSP, model: C11367-11) with a slit width of 3 nm for both excitation and emission. The hydrodynamic size distribution of PEG-DP-PhE FPNs in phosphate buffer saline was also investigated by dynamic laser scattering (DLS), which was conducted on a zetaPlus particle size analyzer (ZetaPlus, Brookhaven Instruments, Holtsville, NY). Gel permeation chromatography (GPC) analyses of polymers were performed using *N*,*N*-dimethyl formamide (DMF) containing 50 mM LiBr as the eluant. The GPC system was a Shimadzu LC-20AD pump system consisting of an auto injector, a MZ Gel SDplus 10.0 μm guard column (50 × 8.0 mm, 10^2 Å) followed by a MZ-Gel SDplus 5.0 μm bead-size column (50–10^6 Å, linear) and a Shimadzu RID-10A refractive index detector.

2.2. Synthesis of CHO-PEG-CHO

The hydrophilic polymer (named as CHO-PEG-CHO) was synthesized through simple esterification reaction (Scheme S2). In brief, PEG (1 g, 1 mmol), CBA (330 mg, 2.2 mmol), DCC (1 g, 5 mmol) and DMAP (61 mg, 0.5 mmol) were dissolved in 20 mL of THF in a round-bottomed flask consisting of a magnetic stir bar. The reaction mixture was heated to 40 °C at nitrogen atmosphere for 24 h. Afterward, the reaction was stopped by pouring the reaction solution into 100 mL of diethyl ether to obtain white precipitates. The precipitates were washed with diethyl ether and dried under vacuum to a constant weight.

2.3. Fabrication of PEG-DP-PhE

The PEG-DP-PhE copolymers were fabricated via a rapid and facile microwave-assisted MCR method. The dye NH_2-PhE-NH_2 (71 mg, 1 mmol), CHO-PEG-CHO (126 mg, 1 mmol) and DP (414 mg, 3 mmol) were added into a round-bottomed flask. The reaction mixture was irradiated in a multimode microwave reactor (MCR-3, China) at 100 °C for only 5 min with drastically string. Upon cooling, the mixture solution was put in the dialysis bag (MWCO 3500) and placed into the fresh water for 48 h, followed by evaporating to obtain the raw product. The product was then purified by dissolving in THF and precipitated in cool diethyl ether for several times. Finally, the obtained PEG-DP-PhE copolymers were dried at vacuum for further characterization.

2.4. Cell viability evaluation

The biocompatibility of materials is a very important property for their application in the biomedical fields. In this work, the biocompatibility of PEG-DP-PhE FPNs was evaluated through