Fluorescent polymeric nanoparticles with aggregation-induced emission feature have been fabricated via combination of chain transfer free radical polymerization and multicomponent reaction

Preparation of PEGylated polymeric nanoprobes with aggregation-induced emission feature through the combination of chain transfer free radical polymerization and multicomponent reaction: self-assembly, characterization and biological imaging applications

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Abstract

Self-assembly of amphiphilic luminescent copolymers is a general route to fabricate fluorescent polymeric microparticles (FPMs). In this work, the FPMs with aggregation-induced emission (AIE) feature were fabricated via the combination of the chain transfer free radical polymerization and “one-pot” multicomponent reaction, which conjugated the aldehyde-containing AIE active dye AIE (CHO-An-CHO) and amino-terminated hydrophilic polymer (ATPPEGMA) using mercaptoacetic acid (MTA) as the “lock” molecule. The structure, chemical compositions, optical properties as well as biological properties of the PPEGMA-An-PPEGMA FPMs were characterized and investigated by means of a series of techniques and experiments in detail. We demonstrated the final copolymers showed amphiphilic properties, strong yellow fluorescence and high water dispersibility. Biological evaluation suggested that PPEGMA-An-PPEGMA FPMs possess low cytotoxicity and can be used for cell imaging. More importantly, many other AIE active FPMs are expected to be fabricated using the similar strategy because of the good substrate and monomer applicability of the multicomponent reaction and chain transfer living radical polymerization. Therefore, we could conclude that the strategy described in this work should be of great interest for fabrication of multifunctional AIE active nanoprobes for biomedical applications.

Key words: Chain transfer free radical polymerization, multicomponent reaction, aggregation-induced emission, fluorescent polymeric nanoparticles, biomedical imaging

1. Introduction

With the drastic development of cancer diagnostic and treatment techniques, facile fabrication of multifunctional fluorescent bioprobes at nanoscale has attracted increasing attention for the applications of biological imaging, disease diagnosis, drug delivery and nanotheranostics.[1-9] Traditional fluorescence probes with intense emission have been frequently emerged based on different fluorescent materials such as fluorescent proteins,[10-12] organic dyes[13-17] and inorganic quantum dots (IQDs) etc.[18-24] However, the malicious drawbacks such as low photobleaching and molar absorptivity were encountered in these above mentioned bioprobes based on fluorescent proteins and organic dyes.[25, 26] meanwhile IQDs also suffered from deathful defects for their long-time application and accumulation in reticuloendothelial system because of their poor biodegradability and high cytotoxicity.[27] These obvious disadvantages have greatly limited the applications of traditional bioprobes. Therefore, exploration of novel fluorescent bioprobes with desirable biocompatibility, strong photobleaching resistance and high fluorescent intensity is highly desirable for development of more effective tools for biological tracking, sensing and diagnostic.

Since the first report of aggregation-induced emission (AIE) phenomenon in 2001 by Tang et al,[28] numerous fluorescent biological probes based on AIE dyes such as siloles,[29-32] triphenylethene,[33-35] cyano-substituted diarylethene,[36-39] distyrylanthracene derivatives,[40, 41] tetraphenylethene[42-45] and phenothiazine[46-49] have been synthesized and used to fabricate bioprobes for cell imaging and detection of heavy metal ions due to their unique AIE properties, strong fluorescence, fluorescent stability, multifunctional potential and low toxicity. Like many other organic dyes, the dyes with AIE feature are also composed with many hydrophobic aromatic rings. This is a great challenge for direct usage of these AIE active dyes in biological systems. In order to overcome the issue for biomedical applications, many efforts have been devoted to prepare high water dispersible and biocompatible fluorescent polymeric microparticles (FPMs) over the past several years. Different strategies based on non-covalent self-assembly, covalent linkage, polymerization and formation of dynamic bonds have been well established.[7, 45, 50-57] However, most of these strategies also show some drawbacks, including lack of functional, poor stability and complexity experimental procedure. Therefore, exploration of a novel method with rapid, high efficiency and eco-friendly for the preparation of FPMs is still desirable.

Similar to the click reaction, multicomponent reaction (MCR) has attracted increasing research

attention in polymer and materials science because of their obvious advantages such as modular, atom-economical, efficient and environmentally friendly.[58-65] The single product with reasonably high yields could be obtained under three or more reactants via MCR. Based on previous reports by Tao et al, who described that efficiently covalent combination of amine and aldehyde substrates using mercaptoacetic acid (MTA) as “lock” via mercaptoacetic acid locking imine (MALI) reaction.[66] The most important is that MALI reaction could be carried out at eco-friendly conditions (without catalysts and room temperature). Based on numerous advantages of MCR, our group reported an attractive strategy with environmentally friendly and high efficiency for fabrication of fluorescent AIE active FPMs via combining chain transfer free radical polymerization and MALI reaction. Synthetic process could be clearly described in follows (**Scheme 1**): **a)** The amine-terminated hydrophilic poly(ethylene glycol) methyl acrylate (ATPPEGMA) was synthesized by chain transfer free radical polymerization using cysteamine hydrochloride as the chain transfer agent and PEGMA as monomer. **b)** The AIE dye (CHO-An-CHO) with AIE active yellow fluorescence was synthesized via three steps reaction. **c)** Based on a facile MCR, the FPMs with strong fluorescence, great water dispersibility and cytocompatibility have been prepared using three components (CHO-An-CHO, ATPPEGMA and MTA) in one-pot at room temperature in a short time. Furthermore, the experiment of cell viability evaluation and cell imaging for synthetic FPMs in this work show their desirable application prospects in biomedical fields.

Scheme 1 The representative schematic routes for the preparation of AIE active FPMs via combining chain transfer free radical polymerization and one-pot MALI reaction. a) Preparation of hydrophilic amino-terminated PPEGMA through chain transfer free radical polymerization. b) The synthesis of AIE dye (CHO-An-CHO) with strong yellow fluorescence based anthracene unit. c) The fabrication of through MCR.

2. Experiment

2.1 Materials and characterization

All chemical agents were of analytical grade and were used as received without any further purification. All aqueous solutions were prepared with doubly distilled water. All the chemicals such as cysteine hydrochloride (98%, MW 175.64 Da), ammonium persulphate (98%, MW 228.2 Da), PEGMA (96%, MW 95 Da), anthracene (98%, MW 178.3 Da), terephthalaldehyde (98%, MW 134.13 Da) and other reagents were purchased from Sinopharm Chemical reagents Co., Ltd. (Shanghai, China).

^1H nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance-400 spectrometer with D_2O and CDCl_3 as the solvents. The synthetic polymers and materials were characterized by Fourier transform infrared spectroscopy (FT-IR) using KBr pellets. The FT-IR spectra were supplied

from Nicolet5700 (Thermo Nicolet corporation). Transmission electron microscopy (TEM) images were recorded on a Hitachi 7650B microscope operated at 80 kV. The TEM specimens were got by putting a drop of the nanoparticle ethanol suspension on a carbon-coated copper grid. The fluorescence data was obtained from the Fluorescence spectrophotometer (FSP, model: C11367-11, Hamamatsu, Japan). The hydrodynamic size distribution of PPEGMA-An-PPEGMA FPMs in phosphate buffered saline (PBS) was determined by dynamic laser scattering (DLS) using ZetaPlus apparatus (Brookhaven Instruments, Holtsville, NY).

2.2 Synthesis AIE dye and its intermediates

The AIE dye (CHO-An-CHO) was synthesized according to our previous report.[67] The detailed experimental procedure and characterization results can be found in supporting information.

2.3 preparation of ATPPEGMA

The hydrophilic polymer was prepared through chain transfer free radical polymerization using PEGMA as monomer and cysteamine hydrochloride as chain transfer agent. The mixture of PEGMA (1.9 g, 2 mmol), ammonium persulphate (45.6 mg, 0.2 mmol) and cysteamine hydrochloride (35 mg, 0.2 mmol) was dissolved in pure water (20 mL) and removed into reaction bulb. The reaction was carried out at nitrogen atmosphere for 24 h. The pure ATPPEGMA was obtained via dialysis with fresh water for 48 h and soaked in ethanol for 6 h and dried at vacuum oven.

2.4 Fabrication of PPEGMA-An-PPEGMA FPMs via MALI reaction

As shown in **Scheme 1c**, the FPMs with strong yellow luminescence were prepared via a facile and atom-economical one-pot method. The synthetic CHO-An-CHO (50 mg), ATPPEGMA (500 mg) and MTA (500 mg) were mixed in reaction bulb with 10 mL THF solution and stirred for 2 h. The fluorescent polymer could be obtained by precipitating with cool diethyl ether and dry at vacuum oven.

2.5 Cell viability examination of PPEGMA-An-PPEGMA FPMs

The cell viability of PPEGMA-An-PPEGMA FPMs on HeLa cells was evaluated by cell counting kit-8 (CCK-8) assay.[68-70] Briefly, cells were put into 96-well microplates at a density of 5×10^4 cells mL^{-1} in 160 μL of cell culture medium containing 10% fetal bovine serum (FBS). After 24 h of cell attachment, the HeLa cells were incubated with different concentrations of PPEGMA-An-PPEGMA FPMs (20-100 $\mu\text{g mL}^{-1}$) for 8 and 24 h. Then cell culture medium containing FPMs were removed and washed with PBS three times. 10 μL of CCK-8 dye and 100 μL of Dulbecco's modified eagle medium (DMEM) were added to each well and incubated for another 2 h at 37 °C. Afterward, plates were

analyzed using a microplate reader (VictorIII, 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 PEG-TPE FONs), which represented 100% CCK-8 reduction. Three replicate wells were used per microplate, and the experiment was operated for three times. Cell survival was expressed as absorbance relative to that of untreated controls. Results are presented as mean $\pm$ standard deviation (SD).

2.5 Confocal microscopic imaging

HeLa cells were cultured in DMEM supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U mL⁻¹ penicillin, and 100 μ g mL⁻¹ of streptomycin. Cell culture was controlled at 37 °C in a similar humidified condition of 95% air and 5% CO₂ in culture medium. Before treatment, cells were seeded in a glass bottom dish with a density of 1 $\times$ 10⁵ cells per dish. On the day of treatment, the cells were incubated with PPEGMA-An-PPEGMA FPMs at a final concentration of 40 μ g mL⁻¹ for 3 h at 37 °C. Afterward, the cells were washed three times with PBS to remove the PPEGMA-An-PPEGMA FPMs and then fixed with 4% paraformaldehyde for 10 min at room temperature. Cell images were obtained using a confocal laser scanning microscope (CLSM) Zesis 710 3-channel (Zesis, Germany) with the excitation wavelength of 458 nm.

3. Result and discussion

In this work, we reported a high-efficiency and fast strategy for the fabrication of AIE active fluorescent bioprobe with strong yellow fluorescence, high water dispersibility and excellent cytocompatibility and bioimaging properties. This strategy mainly relied on the combination of chain transfer free radical polymerization and MALI reaction, which could conjugate the amino group containing compounds and aldehyde containing compounds using MTA as the molecular “lock” in one-pot route. The CHO-An-CHO with AIE active characteristics was synthesized following the synthetic route shown in **Scheme 1b**. The structure of CHO-An-CHO and its intermediates could be confirmed from the ¹H NMR spectra, which was offered in ESI (**Fig. S1-3**). CHO-An-CHO possess hydrophobic nature and difficult to be dissolved in aqueous solution directly. This will largely limited its applications in biomedical fields. One of the effective way to overcome this problem is to fabrication of hydrophobic AIE dyes containing amphiphilic copolymers, which can well disperse in aqueous solution and emit strong luminescence for their AIE feature. The successful conjugation of

CHO-An-CHO with ATPPEGMA could be deduced by ^1H NMR spectra of ATPPEGMA and PPEGMA-An-PPEGMA. As shown in the **Fig. 1**, comparing with ^1H NMR spectrum of ATPPEGMA, a series of new peaks at 7.0-8.6 ppm could be clearly observed. It could be assigned to the signal of aromatic rings, suggesting the successful introduction of CHO-An-CHO into amphiphilic copolymers. Furthermore, the disappearance of peak at 4.78 ppm ($-\text{NH}_2$), while the appearance of new peak at 2.95 ppm ($\text{S}-\underline{\text{CH}_2}-\text{C}(\text{O})$), further confirming the conjugation CHO-An-CHO with ATPPEGMA via MTA as the lock. Therefore, we can preliminary draw a conclusion that successful formation of amphiphilic PPEGMA-An-PPEGMA through MALI reaction.

Fig. 1 The ^1H NMR spectra of the hydrophilic polymer (ATPPEGMA) and PPEGMA-An-PPEGMA. (A) ^1H NMR spectrum of ATPPEGMA. The chemical shift at 4.78 ppm could be attributed to $-\text{NH}_2$. (B) ^1H NMR spectrum of PPEGMA-An-PPEGMA. The inset represents the amplified ^1H NMR spectrum of PPEGMA-An-PPEGMA ranging from 7.0 to 9.0 ppm. A series of peaks ranging from 7.0 ppm to 8.6 ppm was observed. On the other hand, the new peak at 2.93 ppm belongs to the $\text{S}-\underline{\text{CH}_2}-\text{C}(\text{O})$, which was formed by MALI reaction. These results implied that AIE dye CHO-An-CHO was introduced into

copolymers.

The AIE feature of CHO-An-CHO was measured through dissolving of dye in the solution with different fractions of water to THF but with the same dye concentration. As shown in **Fig. 2A**, we studied their photoluminescence behavior when CHO-An-CHO (1 mg) was dissolved in the THF (20 mL) as mother solution. The fluorescence intensity of CHO-An-CHO is weak when dissolved in the THF solution, and the intensity was increased slowly until water fraction arrived 50%. Afterward, the emission intensity was strengthened bristly when the fractions of water were further increased. Furthermore, the emission wavelengths were slightly redshifted from 543 nm to 554 nm along with increase of water fractions. It could be attributed to the aggregation of CHO-An-CHO, demonstrating that CHO-An-CHO possesses unique AIE characteristics. On the other hand, the change trend of fluorescent intensity with water fractions was displayed in **Fig. 2B**. The change of fluorescent intensity was weakened when water fractions arrived 40%. However, the emission intensity was enhanced drastically when water fractions were increased to 90%, suggesting unique AIE feature of CHO-An-CHO.

Fig. 2 (A) fluorescent spectra of CHO-An-CHO in mixture solution of THF/H₂O with different water fractions; $\lambda = 434$ nm. (B) Variation in the fluorescent intensity of CHO-An-CHO in THF/water mixture solution with different water fraction

Due to their amphiphilic properties, PPEGMA-An-PPEGMA copolymers were prone to self-assemble and formed core shell FPMs, in which the hydrophobic AIE dye CHO-An-CHO was encapsulated in the core and hydrophilic PPEGMA expanded in water as the shell. Therefore, we could expect that PPEGMA-An-PPEGMA FPMs should emit strong fluorescence because of the aggregation in the core and AIE feature of CHO-An-CHO. At the same time, the hydrophilic PPEGMA shell could

provide the high water dispersibility of PPEGMA-An-PPEGMA FPMs. The stability of PPEGMA-An-PPEGMA FPMs in water and PBS was examined by DLS. Results showed that the zeta-potential values of PPEGMA-An-PPEGMA FPMs are -26.5 ± 3.4 and -24.5 ± 2.8 mV, respectively. The above results indicated that PPEGMA-An-PPEGMA FPMs possess excellent dispersibility in aqueous solution. These features make PPEGMA-An-PPEGMA FPMs promising for biomedical applications. The self-assembly of PPEGMA-An-PPEGMA copolymers was first evidenced by TEM observation. As shown in **Fig. 3**, the self-assemblies of PPEGMA-An-PPEGMA copolymers displayed spherical morphology and uniform size. As evidenced by TEM image, we can find that the size of PPEGMA-An-PPEGMA FPMs is about 100 nm. Therefore, TEM image could be as powerful evidence for successful conjugation of hydrophobic AIE materials with hydrophilic ATPPEGMA via a rather facile one-pot MALI reaction. Furthermore, the hydrodynamic size distribution of PPEGMA-An-PPEGMA FPMs based on DLS measurement was also provided to compare with the TEM results. We demonstrated that size of PPEGMA-An-PPEGMA FPMs in PBS is 135.7 ± 11.8 nm. This method has extensive application prospect for fabrication of FPMs because of efficient, simple and environmentally friendly.

Fig. 3 TEM image of PPEGMA-An-PPEGMA FPMs dispersed in water, which suggesting the uniform size of PPEGMA-An-PPEGMA FPMs.

Except from the above-mentioned results from TEM and ^1H NMR, FT-IR spectra are also used to confirm the successful synthesis of PPEGMA-An-PPEGMA FPMs. As shown in **Fig. 4A**, two

characteristic peaks were respectively located at 2989 and 2930 cm^{-1} , which can be assigned to the stretching vibration bonds of $-\text{CH}_3$ and $-\text{CH}_2$ of PPEGMA. Furthermore, other peaks ($-\text{NH}_2$ (3439 cm^{-1}), $\text{C}=\text{O}$ (1647 cm^{-1}), $\text{C}-\text{C}$ (1420 cm^{-1}), $\text{C}-\text{O}$ (1078 cm^{-1})) were clearly observed, providing us powerful evidence that preparation of amine-terminated PPEGMA. Compared with FT-IR spectrum of ATPPEGMA, the disappearance of signal at 3439 cm^{-1} demonstrated that three components of ATPPEGMA, CHO-An-CHO and MTA were successfully reacted via a facile one-pot method. Furthermore, the fingerprint region of benzene ring ranging from 500 to 1000 cm^{-1} were discovered, which was attributed to the introduction of AIE dye in PPEGMA-An-PPEGMA copolymers (**Fig. 4B**). This further suggested that successful fabrication of FPMs based on unique AIE dyes by this interesting method.

The UV-Vis absorption spectrum was also utilized to characterize the chemical structure of PPEGMA-An-PPEGMA FPMs. The UV-Vis spectrum was conducted through dispersion of PPEGMA-An-PPEGMA copolymers in pure aqueous solution. As shown in **Fig. 4C**, two absorption peaks were found at 272 and 428 nm. The strong adsorption peak with maximum wavelength at 272 nm could be attributed to $\pi \rightarrow \pi^*$ transition, which suggested that the existence of conjugated chemical structure (e. g. conjugated allene or aromatic rings). On the other hand, a shoulder peak at 314 nm was also found in UV-Vis spectrum. This adsorption peak can be ascribed to the R band of $n \rightarrow \pi^*$ transition, which suggested that hetero atoms such as S, O, and N have conjugated with aromatic rings. Furthermore, the weak adsorption peak at 427 nm implied that the number of conjugated double bonds is greater than 10. The inset of **Fig. 4C** is the optical image of PPEGMA-An-PPEGMA aqueous suspension. It can be seen that PPEGMA-An-PPEGMA can be well dispersed in water. The mark at the background of cuvette can be clearly distinguished. Therefore, we could conclude that the PPEGMA-An-PPEGMA copolymers have been prepared through MALI reaction.

Due to the strong fluorescence of CHO-An-CHO, PPEGMA-An-PPEGMA formed by covalent bond also showed strong yellow fluorescence in water because of the aggregation and self-assembly of amphiphilic materials in aqueous solution. As seen from **Fig. 4D**, it can be noticed that the maximum emission wavelength of PPEGMA-An-PPEGMA FPMs located at 558 nm, while their maximum excitation wavelength located at 434 nm. Well consistent with the fluorescent spectra, strong and uniform yellow fluorescence was observed after irradiation of PPEGMA-An-PPEGMA water suspension by UV lamp at 365 nm (inset of **Fig. 4D**). Therefore, according to the results of absorption

and fluorescent spectra, the FPMs with strong fluorescence, uniform size and great water dispersibility were prepared based on combination of chain transfer free radical polymerization and MALI reaction. Thus synthetic FPMs in this work have great potential for cell imaging applications because of their outstanding features.

Fig. 4 Characterization of ATPPEGMA and PPEGMA-An-PPEGMA FPMs. (A) the FT-IR spectrum of ATPPEGMA, the strong stretching vibration bands of -NH₂ located at 3439 cm⁻¹ was observed, suggesting that successful synthesis of ATPPEGMA; (B) the FT-IR spectrum of PPEGMA-An-PPEGMA FPMs, two new peaks ranging from 500 to 1000 cm⁻¹ were appeared, which could be ascribed to the out-of-plane bending of aromatic ring, demonstrating that successful conjugation of ATPPEGMA with CHO-An-CHO; (C) UV-Vis spectrum of PPEGMA-An-PPEGMA FPMs. The inset used “five-pointed star” as background indicated the great dispersibility of FPMs in water. (D) Fluorescent excitation (Ex) and emission (Em) spectra of PPEGMA-An-PPEGMA FPMs, inset of **Fig. 4D** is optical image of PPEGMA-An-PPEGMA water suspension after irradiated at 365 nm using UV lamp.

To explore the potential biomedical application of PPEGMA-An-PPEGMA FPMs, the possible adverse effect of PPEGMA-An-PPEGMA FPMs to HeLa cells was preliminary evaluated based on CCK-8 assay. As shown in **Fig. S4**, the relationship between cell viability and concentration of

PPEGMA-An-PPEGMA FPMs could be clearly observed. It can be seen that almost no cell viability was obviously decreased when HeLa cells were incubated with 20-100 $\mu\text{g mL}^{-1}$ of PPEGMA-An-PPEGMA FPMs for 8 h. Even the concentrations of PPEGMA-An-PPEGMA FPMs were as high as 100 $\mu\text{g mL}^{-1}$, the cell viability values are still maintained at about 100%. Furthermore, the cell viability values will decrease gradually when the incubation time was increased to 24 h. However, the cell viability values of PPEGMA-An-PPEGMA FPMs towards HeLa cells are still greater than 95%. The results of biocompatibility evaluation provide important information for the potential biomedical applications of PPEGMA-An-PPEGMA FPMs. The cell uptake behavior of PPEGMA-An-PPEGMA FPMs was investigated by CLSM. As displayed in **Fig. 5**, the bright yellow luminescence could be clearly observed within cells after HeLa cells were incubated with 40 $\mu\text{g mL}^{-1}$ of PPEGMA-An-PPEGMA FPMs for 3 h. This indicated that PPEGMA-An-PPEGMA FPMs could be applied in biological imaging due to their intense fluorescence. On the other hand, the areas with relative weak fluorescent intensity or almost no fluorescent signal could be observed at the centre of cells. These areas might be the location of cell nucleus, suggesting that PPEGMA-An-PPEGMA FPMs can be internalized by cells and mainly distributed in cytoplasm. The cell uptake mechanism is possibly ascribed to the cell endocytosis because no active targeting agents were immobilized on the surface of PPEGMA-An-PPEGMA FPMs. That is to say, less PPEGMA-An-PPEGMA FPMs can be used for biological imaging if the surface of PPEGMA-An-PPEGMA FPMs was functionalized with active targeting agents. More importantly, the dosage used for cell uptake experiment is 40 $\mu\text{g mL}^{-1}$, which is much less than the biocompatible dosage of PPEGMA-An-PPEGMA FPMs. More importantly, Mass production of these AIE active FPMs via combining AIE dyes and MALI reaction is possible because of its high-productivity, high-efficiency (short time), easy operation (occured in air, room temperature), environmentally friendly (free catalyst). Therefore, combining the other advantages of PPEGMA-An-PPEGMA FPMs, we could conclude that PPEGMA-An-PPEGMA FPMs are promising biomaterials for biomedical applications.

Fig. 5 CLSM imaging of HeLa cells incubated with $40\ \mu\text{g mL}^{-1}$ of PPEGMA-An-PPEGMA FPMs for 4 h. (A) fluorescent imaging under the irradiation with 458 nm laser, (B) bright field, (C) merged image of A and B. Scale bar = 20 μm .

4. Conclusion

In summary, we developed a simple and effective strategy to fabricate AIE active FPMs based on the chain transfer free radical polymerization and a one-pot MALI reaction. The successful formation of amphiphilic AIE dye containing copolymers was confirmed by different characterization techniques in detail. Due to the hydrophobic AIE dye (CHO-An-CHO) was aggregated in the core while hydrophilic polymers (ATPPEGMA) was covered on the shell, thus obtained amphiphilic fluorescent materials are readily self-assembled into stable nanoparticles in aqueous solution. The evaluation of cell viability and CLSM images could provide powerful evidence for wide biomedical application prospect of PEGMA-An-PEGMA FPMs prepared in this work. Comparing with other fluorescence polymeric nanoparticles, the constructed AIE-active FPMs in this work emit strong fluorescence, well dispersed in aqueous solution and excellent biocompatibility, which could resolve the problems of aggregated toxicity from inorganic fluorescent quantum dots, low stokes shift from fluorescent proteins and ACQ effect from conventional organic small dyes. Furthermore, not only these unique properties such as uniform size, intense fluorescence, great water dispersibility and biocompatibility make them great potential candidates for biomedical diagnosis and tracking, but also this facile method described in this work is meaningful for fabricating many other functional AIE active FPMs, which could be succinctly summarized in follows. Firstly, the amino-terminated PPEGMA could be replaced by other amino-terminated functional polymers due to the good applicability of chain transfer free radical polymerization. Secondly, the high-efficiency, facile and environmentally friendly of this method makes the mass production of FPMs possible. Therefore, combining all these attractive advantages of FPMs and one-pot strategy, more and more multifunctional fluorescent nanoparticles would be prepared and follow resulting far-reaching positive influence in biomedical field.

Acknowledgements

This research was supported by the National Science Foundation of China (Nos. 51363016, 21474057, 21564006, 21561022), and the National 973 Project (Nos. 2011CB935700), and the innovation fund of

graduate student of Nanchang University.

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