Polymer Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/polymers

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

PAPER

Amphiphilic Fluorescent Copolymers *via* One-pot Combination of Chemoenzymatic Transesterification and RAFT Polymerization: Synthesis, Self-assembly and Cell Imaging

Zengfang Huang,*[ab] Xiqi Zhang,[b] Xiaoyong Zhang,[b] Changkui Fu,[b] Ke Wang,[b] Jinying Yuan,[b] Lei Tao*[b] and Yen Wei*[b]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

The development of fluorescent organic nanoparticles (FONs) based on aggregation induced emission (AIE) dyes has attracted great research attention in recent years. In this work, a novel one-pot strategy for the fabrication of AIE-based FONs was developed *via* combination of RAFT polymerization and enzymatic transesterification for the first time. During this procedure, a hydrophobic tetraphenylethene-functionalized AIE dye (named as TPEOH) with a hydroxyl end functional group and a hydrophilic polyethylene glycol monomethyl ether (mPEG-OH, M_n = 350) were simultaneously linked onto the methacrylate monomer by the enzymatic transesterification. The amphiphilic copolymer by RAFT polymerization of functional methacrylate monomers tended to self-assemble into FONs with the hydrophobic AIE core coved by hydrophilic PEG shell, and the molar fraction of TPE and PEG in the polymer was about 30.5% and 69.5%, respectively, with 4700 g mol^{-1} (M_n) and a narrow polydispersity index (PDI) (~1.30). The obtained amphiphilic polymer nanoparticles (named as TPE-PEG) presented good fluorescent performance and excellent dispersibility in aqueous solution. More importantly, these FONs showed spherical morphology, uniform size (about 200 nm), and excellent biocompatibility, making them promising for bioimaging applications.

1. Introduction

In the last decade, fluorescent organic nanoparticles (FONs) have attracted increasing interest for their high potential in biology and biochemistry, and are especially attractive for sensing, imaging, and biomedical applications.[1-4] Compared with the conventional fluorescent inorganic nanoparticles including carbon dots, quantum dots, and fluorescent silicon dots, FONs have many advantages such as low toxicity, biocompatibility, biodegradability, and adjustable functionalities.[5-9] However, most of the organic fluorophores have inherent hydrophobic features and are insoluble in the water solution. Therefore, the design and fabrication of amphiphilic FONs to improve their hydrophilic feature has attracted considerable attention in the fields of bioengineering applications. Recently, the FONs based on aggregation-induced emission (AIE) material with good water solubility and biocompatibility have been facilely prepared by mixing AIE dye (An18) and F127 surfactant.[10] The results demonstrated that such AIE-based FONs were biocompatible with cells and could be easily observed in cells. However, thus obtained FONs showed poor stability in the water solution, and a lot of small organic spheres which were aggregated into large nanoparticles with diameters ranging from 400 to 600 nm were observed. On the other hand, this non-covalent strategy has also encountered some problems, such as the fluorophores might leak out of the particles with time. Thus, it is highly necessary for introducing some covalent strategies into the fabrication of AIE-based FONs. Furthermore, the design of unique morphologies and controlled dimensions at nanoscale is of critical importance for various technological applications such as bioimaging and drug delivery.[11-14] Polyethylene glycol (PEG) is a type of hydrophilic polymer, which has been approved by the US Food and Drug Administration (FDA). Owing to its good biocompatibility, low immunogenicity, and high water solubility, PEG has been regarded as one of the best choices for surface modification of both inorganic and organic nanoparticles.[15] When a polymerizable AIE dye was employed into the polymerization of an amphiphilic monomer poly(ethylene glycol) monomethacrylate (PEGMA) through RAFT polymerization, the obtained amphiphilic polymers can be readily self-assembled into AIE-based FONs between 100 and 200 nm and exhibit high water dispersibility, intense fluorescence, and excellent biocompatibility.[16] We previously reported a red cross-linkable R-PEG FONs *via* polymerization of cross-linkable AIE dye (R-E) and PEGMA through RAFT polymerization. Thus obtained polymers with amphiphilic properties tended to self-assemble into uniform FONs with diameters of about a few hundred nanometers and showed uniform size, high water dispersibility, strong red fluorescence, and excellent biocompatibility.[17] A novel

[a]*College of Chemistry and Biology，Zhongshan Institute, University of Electronic Science & Technology of China，Zhongshan, 528402, P. R. China. hzf105@163.com*
[b]*Department of Chemistry, the Tsinghua Center for Frontier Polymer Research, Tsinghua University, Beijing 100084, P. R. China. E-mail: leitao@mail.tsinghua.edu.cn; weiyen@tsinghua.edu.cn*

method has been further developed to fabricate $PhNH_2$-OA-PEG FONs through one-pot ring-opening polymerization and condensation reaction at room temperature and in air without needing catalysts or initiators.[18-19] These obtained FONs exhibited high water dispersibility, strong fluorescence, and excellent biocompatibility, making them promising for bioimaging applications.

Due to the excellent tolerance to many functional monomers and a large range of solvents, reversible addition-fragmentation chain transfer (RAFT) polymerization, as a metal-free approach, has been regarded as a powerful tool to facilely prepare various functional polymers with desired molecular weights, architectures, and narrow polydispersity indices (PDIs).[20-26] Enzymes are regarded as green and efficient catalysts, and have been extensively used in organic synthesis.[27] Our group has investigated the enzymatic transesterification of 2,2,2-trifluoethyl methacrylate (TFEMA) and alcohols (R) with ATRP and RAFT polymerization,[28-29] and the results indicated that the reaction rate of transesterification was very fast compared with the controllable polymerization, and the fraction of (R)-OMA product of transesterification in the polymer was proved more than 86%.

The combination of different (catalytic) reactions into a one-pot system could not only avoid tedious intermediate purification steps, but also provide a powerful and exquisite strategy for sophisticated polymer synthesis and modification.[30-34] Tetraphenylethene (TPE) derivative is a class of well-known AIE material, and has been widely developed for chemosensor and biomedical applications.[35-37] In this contribution, a novel one-pot strategy of smart combination of RAFT polymerization and in situ enzymatic monomer transesterification for fabrication of AIE-based FONs was developed for the first time. Novozym435 was used to catalyze the transesterification between acyl donor monomer TFEMA and primary AIE-based alcohols (TPEOH) or mPEG-OH to form target monomer R (meth)-acrylate (RMA) of TPEMA and PEGMA. Such obtained new monomers RMA subsequently participated in RAFT polymerization to obtain a new amphiphilic polymer with transformed side groups, which tended to self-assemble into FONs in aqueous solution. To study the cell imaging application, the dispersibility, AIE property, and biocompatibility of the as-prepared polymer were subsequently investigated.

2. Experimental

2.1. Materials and characterization

2,2,2-Trifluoethyl methacrylate (TFEMA，J&K Chemical, 98%), 2,2'-azobisisoheptonitrile (AVBN, J&K Chemical, 98%), triethylamine (TEA, J&K Chemical, 99.5%), polyethylene glycol monomethyl ether (mPEG-OH, J&K Chemical, 98%), and immobilized Candida Antarctica lipase B (Novozym435, Beijing Cliscent Science and Technology Co., LTD) were used as purchased. The tetraphenylethene-functionalized methanol (AIE dye, TPEOH) and the chain transfer agent (CTA) of 4-cyano-4-(ethylthiocarbonothioylthio) pentanoic acid were synthesized according to the literature[38, 39].

Gel permeation chromatography (GPC) analysis of polymer was performed using tetrahydrofuran (THF) as the eluent. The GPC system was a Shimadzu LC-20AD pump system comprising an auto injector, a MZ-Gel SDplus10.0 μm guard column (50 × 8.0 mm, 10^2A) followed by three MZ-Gel SDplus10.0 μm bead-size columns (10^5, 10^3, and 10^2 A) and a differential refractive index (RI) detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10^6 g mol^{-1}. ^{1}H NMR spectrum was obtained using a JEOL JNM-ECA400 (400MHz) spectrometer with tetramethylsilane (TMS) as a reference. UV-vis absorption spectrum was recorded on a Perkin-Elmer LAMBDA 35 UV/vis system. Fluorescence spectra were measured on a PE LS-55 spectrometer with a slit width of 3 nm for the emission of TPE-PEG in water or THF solution. The FT-IR spectra were obtained in a reflection mode on a Perkin-Elmer Spectrum 100 spectrometer (Waltham, MA, USA). Typically, 4 scans at a resolution of 1 cm^{-1} were accumulated to obtain one spectrum. Transmission electron microscopy (TEM) image was recorded on a JEM-1200EX microscope operated at 100 kV, the TEM specimens were made by placing a drop of the TPE-PEG suspension on a carbon-coated copper grid. The critical micelle concentration (CMC) was obtained using a JK99C full-automatic tensiometer through the surface tension versus the different concentration of TPE-PEG.

2.2. One-pot synthesis of fluorescent polymer

The procedure for the one-pot synthesis of fluorescent polymer was as follows. TFEMA (0.144 g, 0.862 mmol), TPEOH (0.100 g, 0.275 mmol), mPEG-OH (0.205 g, 0.587 mmol), TEA (0.087 g, 0.862 mmol), CTA (4.7 mg, 0.018 mmol), AVBN (1.2 mg, 0.005 mmol) and toluene (1.5 mL) were introduced in schlenk tube A and purged by nitrogen flow for 30 min. While Novozym435 (0.079 g) were added into another schlenk tube B equipped with a magnetic stir bar followed by evacuated and backfilled with nitrogen for three times. Then the solution in tube A was cannulated into tube B under nitrogen atmosphere. The final reaction mixture was put into an oil bath maintained at 55 °C for 24 h. At the end of the polymerization, the mixture was centrifuged at 9000 rpm for 10 min to remove immobilized enzyme. The purified polymer was obtained *via* precipitation from THF to petroleum ether for three times, and then dried under vacuum for further characterization, yield: 0.12 g. Finally, 10 mg TPE-PEG polymer was added to 10 mL H_2O, then shaken until it had been dissolved completely, which was used to investigated its self-assembly in H_2O solvent.

2.3. Cytotoxicity of TPE-PEG FONs

Cell morphology was observed to examine the effects of TPE-PEG FONs to A549 cells.[40-41] 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 TPE-PEG FONs prepared in 10% FBS containing media for 24 h. Then all samples were washed with PBS three times to remove the uninternalized FONs. The morphology of cells was observed by using an optical microscopy (Leica, Germany), the overall magnification was ×100.

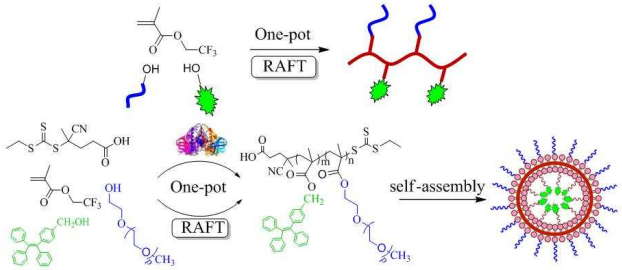
The cell viability of TPE-PEG FONs on A549 cells was evaluated by cell counting kit-8 (CCK-8) assay based on our

previous reports.[42] 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} TPE-PEG 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 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 (Victor III, 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 TPE-PEG FONs), 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 TPE-PEG FONs

The cell uptake of TPE-PEG FONs was evaluated by confocal microscopic imaging.[43-44] Briefly, 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 TPE-PEG FONs 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 TPE-PEG FONs and then fixed with 4% paraformaldehyde for 10 min at room temperature. Cell images were taken with a confocal laser scanning microscope (CLSM) Zesis 710 3-channels (Zesis, Germany) with the excitation wavelength of 405 nm.

3. Results and discussion



Scheme 1 Schematic showing TPE-PEG copolymer self-assembly and its one-pot synthesis of through enzymatic transesterification and RAFT polymerization.

Herein, we reported a straightforward one-pot synthetic strategy through the combination of *in situ* enzymatic monomer transformation and RAFT polymerization. Novozym435 was employed to catalyze the transesterification between acyl donor monomer TFEMA and primary alcohols TPEOH or mPEG-OH to form the respective target monomer R_1 (TPE)-methacrylate (TPEMA) and R_2 (PEG)-methacrylate (PEGMA). The as-prepared new monomers TPEMA and PEGMA subsequently participated in RAFT polymerization to obtain a new polymer with transformed side groups. The introduction of TPEOH would endow the polymer with fluorescence, while the obtained polymer also had good water solubility due to hydrophilic PEG chains, thus the obtained water-soluble fluorescent polymer was expected to be self-assembled into nanoparticles and internalized by cell. The synthetic strategy in current report was schematically illustrated in Scheme 1.

The weight average molecular (M_n) of the final obtained polymer was about 4700 g mol^{-1} with a narrow PDI (~1.30, Fig. 1A). Our previous results exhibited that the molecular weights of polymer by one-pot strategy of RAFT polymerization and transesterification of TFEMA with *n*-hexanol increased linearly with monomer conversions and all the polymers during polymerization remained narrow molecular weight distribution, moreover, the RAFT polymerization exhibited a linear pseudo-first-order kinetic plot versus time, indicative of a controllable RAFT process.[29] The ^{1}H NMR spectrum of the as-prepared polymer is also shown in Fig. 1B. The ester groups of poly-(TFEMA) appeared at 4.32 ppm,[28] and the absence of this peak indicated that the transesterification reaction was nearly complete. The peaks corresponding to the ester groups of poly-(TPE) and poly-(PEG) appeared at 4.84 ppm and 4.05 ppm, respectively. According to the integral ratio of the peaks at 4.84 ppm and 4.05 ppm, the molar fraction of TPE and PEG in the polymer could be calculated as approximate 30.5% and 69.5%, respectively.

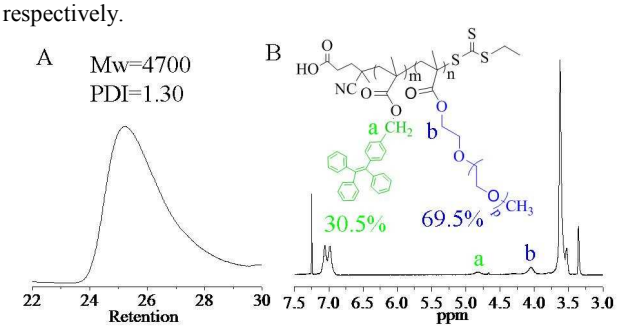


Fig. 1 (A) The GPC trace (THF) and (B) ^{1}H NMR spectrum ($CDCl_3$) of the final obtained fluorescent polymer.

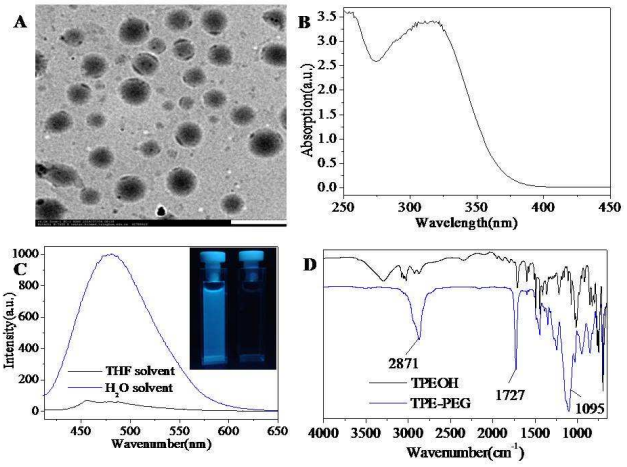


Fig. 2 Characterization of TPEOH and TPE-PEG FONs: (A) TEM image of TPE-PEG FONs dispersed in water, scale bar=500 nm; (B) UV-Vis spectrum of TPE-PEG dispersed in water; (C) Fluorescence emission spectra of TPE-PEG FONs, inset is the fluorescent image of TPE-PEG FONs taken at 365 nm UV light(left bottle (in water), right bottle (in THF)); (D) FT-IR spectra of TPEOH and TPE-PEG, stretching vibration bands of –OH of TPEOH located at 3300 cm^{-1}, strong stretching vibration bands of $–CH_3$ and $–CH_2–$ located at 2871 cm^{-1}, strong stretching vibration bands of C=O located at 1727 cm^{-1} and C–O stretching vibration bands located at 1095 cm^{-1} were observed in the sample of TPE-PEG, suggesting TPE-PEG polymer were formed.

Fig. 2 shows the characterization information of the obtained TPE-PEG FONs. Transmission electron microscopy (TEM) image of Fig. 2A showed that TPE-PEG copolymer could self-assemble into spherical nanoparticles with about 200 nm diameters, which was further confirmed by DLS, and the results indicated that the size was also about 200 nm. The self-assembly of the polymers into spherical nanoparticles further demonstrated that TPEOH and mPEG-OH were successfully incorporated into the polymers *via* RAFT polymerization. The UV absorption spectrum of TPE-PEG FONs dispersed in water was shown in Fig. 2B, and the maximum absorption wavelength was located at 340 nm. The TPE-PEG FONs had the π-π conjugated structure, as the absorption peak of 340 nm might contribute to the electron transition of π→π*. The light scattering or Mie effect of the nanoparticle suspensions in the solution effectively decreased light transmission and caused the apparent high absorption and levelling-off of the tail in the visible region.[45] It was noteworthy that the entire spectrum showed no absorption until the absorption wavelength decreased to 380 nm, which was different with our previous result,[46] indicating that the excellent dispersibility of TPE-PEG FONs in water. Due to the amphiphilic properties of TPE-PEG copolymer, these copolymer tended to self-assemble into nanoparticles in aqueous solution, forming PEGylated AIE-based FONs. Because of the surface of TPE-PEG polymer covered with hydrophilic PEG, the obtained FONs was transparent and showed high stability in pure water solution. On the other hand, the hydrophobic AIE dye (TPE) in the core of FONs would partly aggregate in aqueous solution, however, the obtained TPE-PEG polymer could well dissolve in some organic solvent. As shown in Fig. 2C, the fluorescent property was obviously observed along with the maximum emission peak at 480 nm in the water solution, while in the THF solution, almost no fluorescence was observed, demonstrating the obvious AIE property. A possible explanation for this phenomenon is that in the organic solutions, the intramolecular rotation is active, which serves as a relaxation channel for the excited state to decay, while in the aggregates, the rotation is restricted due to the physical constraint, which blocks the non-radiative path and activates the radiative decay.[47] The FT-IR spectra of TPEOH and TPE-PEG polymer were shown in Fig. 2D. A series of absorbance bands of TPEOH located between 1400 and 1600 cm^{-1} assigned to the stretching vibration of the polycyclic aromatic rings of TPEOH could be observed. The -OH band located at 3300 cm^{-1} could also be obviously observed in the spectrum of TPEOH. As compared with the spectrum of TPEOH dye, the peak of TPE-PEG copolymer located at 3300 cm^{-1} was almost disappeared, indicating the successful incorporation of TPE into the polymers. On the other hand, one characteristic peak located at 1095 cm^{-1} (stretching vibration of C-O) emerged in the spectra of TPE-PEG polymer, further confirming the successful incorporation of PEG into the polymers. In the effect of hydrophobic TPE and hydrophilic PEG, the amphiphilic TPE-PEG copolymer easily tended to self-assemble into uniform FONs in the water solution with the hydrophobic TPE core covered by the hydrophilic PEG.

To investigate the biomedical application of TPE-PEG FONs, the biocompatibility was evaluated by cell incubation with different concentrations of FONs for up to 24 h (Fig. 3A-C). The optical microscopy observations demonstrated that cells maintained their normal morphology. Even when the concentration of TPE-PEG FONs was as high as 80 μg mL^{-1}, the cell morphology didn't change obviously. These preliminary results implied the good biocompatibility of TPE-PEG FONs. To further confirm the good biocompatibility of TPE-PEG FONs, their cell viability to A549 cell by cell counting kit-8 (CCK-8) assay was shown in Fig. 3D. The result indicated that the values of cell viability were more than 90% with 10-120 μg mL^{-1} of TPE-PEG FONs incubated to the cell and no very obvious decrease of cell viability was observed even when the concentration of TPE-PEG FONs increased to 120 μg mL^{-1}. All the above results indicated that TPE-PEG FONs had good biocompatibility with cells and were promising for biomedical applications. From the surface tension versus the different concentration of TPE-PEG, its CMC is about 18.4 μg/mL, thus it is possible of TPE-PEG to dissolve into H_2O in molecule status under 10 μg/mL concentration but keep its sphere structure under 20-120 μg/mL concentration.

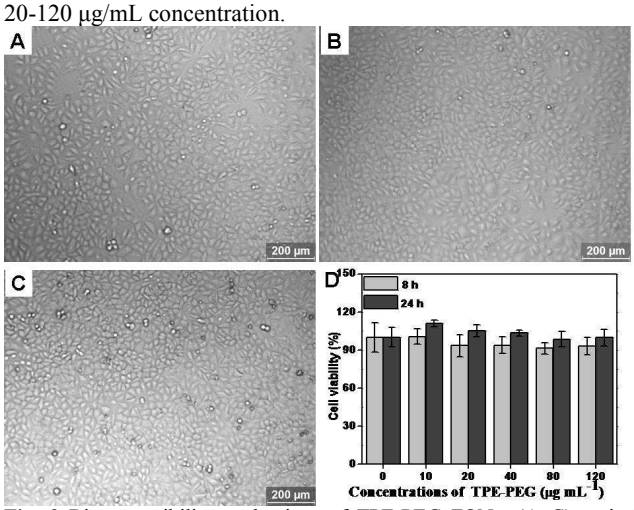


Fig. 3 Biocompatibility evaluations of TPE-PEG FONs. (A–C) optical microscopy images of A549 cells incubated with different concentrations of TPE-PEG FONs for 24 h, (A) control cells, (B) 20 μg mL^{-1}, (C) 80 μg mL^{-1}, (D) cell viability of TPE-PEG FONs with A549 cells. The biocompatibility evaluation suggested that TPE-PEG FONs were biocompatible enough for biomedical applications.

Owing to the excellent water dispersibility, excellent fluorescent feature and favourable biocompatibility, the potential cell imaging application of TPE-PEG FONs was further investigated based on the cell uptake behavior of TPE-PEG FONs by CLSM as shown in Fig. 4A-C. The strong fluorescence at the cytoplasm could be clearly observed after the cell was incubated with 20 μg mL^{-1} of TPE-PEG FONs for 3 h. Furthermore, the areas with relatively weak fluorescence intensity should be the location of the cell nuclei (Fig. 4B).[17] These preliminary results suggested that TPE-PEG FONs could be facilely taken up by cells and mainly located at the cytoplasm. In consideration of the size of FONs and nucleus pore,[16] we believed that TPE-PEG FONs could not enter into the cell nuclei directly. Based on the cell viability results, we believe that TPE-PEG FONs, taking advantage of the merits of AIE and PEG, are biocompatible enough for bioimaging applications. More importantly, owing to the controllability of RAFT polymerization and enzymatic transesterification of TFEMA and AIE dyes, novel monomers by transesterification can also be facilely integrated into polymer nanoparticles, and thus multifunctional imaging and theranostic

platforms can be obtained by controllable polymerization associated with transesterification of TFEMA and AIE dyes. Given the excellent properties, these FONs should be promising for biomedical applications.

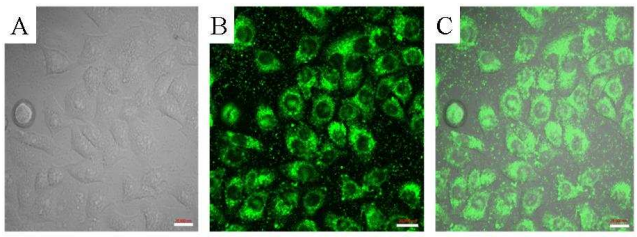


Fig. 4 CLSM images of A549 cells incubated with 20 μg mL^{-1} of TPE-PEG FONs. (A) bright field, (B) excited with a 405 nm laser, (C) merged image of (A) and (B). Scale bar = 20 μm.

4. Conclusions

In summary, we have developed a novel one-pot method for the preparation of TPE-PEG FONs through controllable RAFT polymerization and enzymatic transesterification of TFEMA, functional AIE dye (TPEOH), and a widely used biomedical material (PEGOH). The M_n of such obtained polymers was about 4700 g mol^{-1} with a narrow PDI (~1.30), and the molar fraction of TPE and PEG in the polymer was about 30.5% and 69.5%, respectively. In the water solution, these amphiphilic polymers tended to self-assemble into FONs, with the AIE dye as the hydrophobic core and PEG as the hydrophilic shell. Owing to their surfaces covered with PEG, TPE-PEG FONs showed excellent water dispersibility. Such FONs also showed excellent fluorescence in the aqueous solution owing to the introduction of AIE component. Biocompatibility evaluation suggested that TPE-PEG FONs were biocompatible enough for bioimaging applications. In consideration of the controllability of RAFT polymerization and the convenience of transesterification, numerous AIE based FONs can also be prepared *via* different AIE dyes. Further conjugated with other components such as drugs, imaging agents, and targeting agents, various multifunctional FONs can also be easily fabricated for bioimaging applications. In a word, the strategy combining RAFT polymerization and enzymatic transesterification technology provides an economical and efficient avenue for the fabrication of numerous AIE-based FONs, which are promising for various biomedical applications.

Acknowledgements

This research was supported by the National Science Foundation of China (Nos. 21474057, 21104039, 21134004, 51363016), and the National 973 Project (Nos. 2011CB935700), the natural science foundation of guangdong province (S2013010013580)

References

1 N. Rosi and C. Mirkin, *Chem. Rev.*, 2005, 105, 1547-1562.
2 A. Louie, *Chem. Rev.*, 2010, 110, 3146-3195.
3 J. Hu and S. Liu, *Macromolecules*, 2010, 43, 8315-8330.
4 C. Grazon, J. Rieger, R. Méallet-Renault, G. Clavier and B. Charleux, *Macromol. Rapid Commun.*, 2011, 32, 699-705.
5 Y. Ho and K. Leong, *Nanoscale,* 2010, 2, 60-68.
6 S.Chandra, P. Das, S. Bag, D. Laha and P. Pramanik, *Nanoscale*, 2011, 3, 1533-1540.
7 A. Shiohara, S. Prabakar, A. Faramus, C. Hsu, P. Lai, P. Northcote and R. Tilley, *Nanoscale*, 2011, 3, 3364-3370.
8 A. Vollrath, S. Schubert and U. Schubert, *J. Mater. Chem. B*, 2013, 1, 1994-2007.
9 X. Zhang, X. Zhang, B. Yang and Y. Wei, *Chinese J. Polym. Sci.*, 2014, 32, 871-879.
10 X. Zhang, X. Zhang, S. Wang, M. Liu, L. Tao and Y. Wei, *Nanoscale*, 2013, 5, 147-150.
11 M. Semsarilar, E. Jones, A. Blanazs and S. Armes, *Adv. Mater.*, 2012, 24, 3378-3382.
12 V. Ladmiral, M. Semsarilar, I. Canton and S. Armes, *J. Am. Chem. Soc.*, 2013, 135, 13574-13581.
13 J. Madsen, I. Canton, N. Warren, E. Themistou, A. Blanazs, B. Ustbas, X. Tian, R. Pearson, G. Battaglia and A. Lewis, *J. Am. Chem. Soc.*, 2013, 135, 14863-14870.
14 X. Zhang, X. Zhang, L. Tao, Z. Chi, J. Xu and Y. Wei, *J. Mater. Chem. B*, 2014, 2, 4398-4414.
15 X. Zhang, J. Hui, B. Yang, Y. Yang, D. Fan, M. Liu, L. Tao and Y. Wei, *Polym. Chem.*, 2013, 4, 4120-4125.
16 X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen and Y. Wei, *Polym. Chem.*, 2014, **5**, 356-360.
17 X. Zhang, M. Liu, B. Yang, X. Zhang, Z. Chi, S. Liu, J. Xu, and Y. Wei, *Polym. Chem.*, 2013, 4, 5060-5064.
18 X. Zhang, X. Zhang, B. Yang, J. Hui, M. Liu, W. Liu, Y. Chen and Y. Wei, *Polym. Chem.*, 2014, 5, 689-693.
19 X. Zhang, X. Zhang, B. Yang, J. Hui, M. Liu, Z. Chi, S. Liu, J. Xu and Y. Wei, *Polym. Chem.*, 2014, 5, 683-688.
20 D. Quémener, T. Davis, C. Barner-Kowollik and M. Stenzel, *Chem. Commun.*, 2006, 42, 5051-5053.
21 M. Kade, D. Burke and C. Hawker, *J. Polym. Sci., Part A: Polym. Chem.*, 2010, 48, 743−750.
22 H. Li, B. Yu, H. Matsushima, C. Hoyle and A. Lowe, *Macromolecules*, 2009, 42, 6537-6542.
23 C. Becer, R. Hoogenboom and U. Schubert, *Angew. Chem., Int. Ed.*, 2009, 48, 4900-4908.
24 M. Li, P. De, S. Gondi and B. Sumerlin, *Macromol. Rapid Commun.*, 2008, 29, 1172-1176.
25 C. Boyer, V. Bulmus, T. Davis, V. Ladmiral, J. Liu and S. Perrier, *Chem. Rev.* 2009, 109, 5402-5436.
26 S. Perrier and P. Takolpuckdee, *J. Polym. Sci., Part A: Polym. Chem.*, 2005, 43, 5347-5393.
27 K. Koeller and C. Wong, *Nature*, 2001, 409, 232-240.
28 C. Fu, C. Zhu, S. Wang, H. Liu, Y. Zhang, H. Guo, L. Tao and Y. Wei, *Polym. Chem.*, 2013, 4, 264-267.
29 S. Wang, C. Fu, Y. Zhang, L. Tao, S. Li and Y. Wei, *ACS Macro. Lett.*, 2012, 1, 1224-1227.
30 J. Geng, J. Lindqvist, G. Mantovani and D. Haddleton, *Angew. Chem., Int. Ed.*, 2008, 47, 4180-4183.
31 P. Golas and K. Matyjaszewski, *Chem. Soc. Rev.*, 2010, 39, 1338-1354.
32 L. Mespouille, M. Vachaudez, F. Suriano, P. Gerbaux, O. Coulembier, P. Degée, R. Flammang and P. Dubois, *Macromol. Rapid Commun.*, 2007, 28, 2151-2158.
33 B. van As, P. Thomassen, B. Kalra, R. Gross, E. Meijer, A. Palmans and A. Heise, *Macromolecules*, 2004, 37, 8973-8977.
34 F. Wolf, N. Friedemann and H. Frey, *Macromolecules*, 2009, 42, 5622-5628.
35 X. Zhang, Z. Chi, H. Li, B. Xu, X. Li, W. Zhou, S. Liu, Y. Zhang and J. Xu, *Chem.- Asian J.*, 2011, 6, 808-811.
36 X. Zhang, Z. Chi, H. Li, B. Xu, X. Li, S. Liu, Y. Zhang and J. Xu, *J. Mater. Chem.*, 2011, 21, 1788-1796.
37 X. Zhang, Z. Chi, B. Xu, C. Chen, X. Zhou, Y. Zhang, S. Liu and J. Xu, *J. Mater. Chem.*, 2012, 22, 18505-18513.
38 Y. Park, H. Ahn, B. Canturk, S. Jeon, S. Lee, H. Kang, G. Molander and J. Ham, *Org. Lett.*, 2008, 10, 1215-1218.

39 L. Tao, J. Liu and T. Davis, *Biomacromolecules*, 2009, 10, 2847-2851.
40 X. Zhang, J. Yin, C. Peng, W. Hu, Z. Zhu, W. Li, C. Fan and Q. Huang, *Carbon*, 2011, 49, 986-995.
41 X. Zhang, M. Liu, B. Yang, X. Zhang and Y. Wei, *Colloids and Surfaces B: Biointerfaces*, 2013, 112, 81-86.
42 X. Zhang, H. Qi, S. Wang, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Toxicol. Res.*, 2012, 1, 201-205.
43 X. Zhang, S. Wang, C. Fu, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Polym. Chem.*, 2012, 3, 2716-2719.
44 B. Yang, Y. Zhang, X. Zhang, L. Tao, S. Li and Y. Wei, *Polym. Chem.*, 2012, 3, 3235-3238.
45 X. Zhang, Z. Chi, J. Zhang, H. Li, B. Xu, X. Li, S. Liu, Y. Zhang and J. Xu, *J. Phys. Chem. B*, 2011, 115, 7606-7611.
46 X. Zhang, X. Zhang, B. Yang, J. Hui, M. Liu, Z. Chi, S. Liu, J. Xu and Y. Wei, *Polym. Chem.*, 2014, 5, 318-322.
47 Y. Hong, J. Lam and B. Tang, *Chem. Commun.*, 2009, 4332-4353.

Graphical abstract image

A novel one-pot strategy for the fabrication of AIE-based FONs was developed *via* combination of RAFT polymerization and enzymatic transesterification for the first time, and these FONs showed uniform spherical morphology and excellent biocompatibility, making them promising for bioimaging applications.