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PAPER

One-pot Synthesis and Biological Imaging Application of Amphiphilic Fluorescent Copolymer *via* Combination of RAFT Polymerization and Schiff Base Reaction

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In recent years, fluorescent organic nanoparticles (FONs) based on aggregation induced emission (AIE) dyes have received increasing interest for their potential in biology and biochemistry. In this contribution, a novel one-pot method for the fabrication of AIE-based FONs was developed *via* combination of reversible addition−fragmentation chain-transfer (RAFT) polymerization and Schiff base reaction for the first time. During this procedure, an aldehyde functionalized hydrophobic tetraphenylethene AIE dye (named as TPEA) reacted with the amine group of an amino-ended methacrylamide monomer by Schiff base reaction, and the vinyl group in the monomer synchronously participated in RAFT polymerization together with a PEGMA monomer to form a new fluorescent copolymer. The as-prepared copolymer tended to self-assemble into FONs with the hydrophobic AIE core covered by hydrophilic PEG shell, and the molar fraction of TPEA and PEG in the copolymer was about 20% and 80%, respectively, with 29200 g mol^{-1} (M_n) and a narrow polydispersity index (PDI) (~1.30). The prepared amphiphilic copolymer nanoparticles (named as TPEA-PEG) exhibited good fluorescent feature and excellent dispersibility in water solution. More importantly, these FONs presented spherical morphology, uniform size (~100 nm), and excellent biocompatibility, making them promising for bioimaging applications.

1. Introduction

The combination of different (catalytic) reactions into a one-pot system can not only avoid tedious intermediate purification steps, but also provide a powerful and exquisite strategy for sophisticated polymer synthesis and modification.[1-5] Therefore, one-pot synthetic strategies have attracted increasing academic and industrial interests in polymer chemistry.[6-7] For example, our group has developed a simultaneous one-pot synthesis methodology of functional polymers by combining three orthogonal reactions, namely click reaction, lipase-catalyzed transesterification and atom transfer radical polymerization (ATRP). These reactions were compatible and cooperated well in a one-pot fashion, leading to well-defined new polymers with controllable molecular weights, compositions and functionalities.[8] In our previous report, almost neat Biginelli functionalized homopolymer has been successfully achieved in a one-pot fashion combining the Biginelli reaction and the RAFT polymerization.[9] One-pot strategy to fabricate the thermal responsive quantum dots (QDs)-poly(N-isopropylacrylamide) (PNIPAM) hybrid fluorescent microspheres has also been developed through 3-mercaptopropionic acid (MPA) capped CdTe QDs copolymerizing into PNIPAM microspheres during the monomer polymerization for the first time.[10] Another one-step route to generate fluorescent functionalized carbon nanoparticles (F-CNPs) has been also developed by a hydrothermal method using different carbon sources with specific functional groups. The F-CNPs with diameters from 5 to 30 nm possess high photoluminescence efficiency and are relatively monodispersed.[11] From a hydrophilic poly(ethylene oxide)-block-poly(acrylic acid) (PEO-*b*-PAA) macromolecular RAFT agent which is block-extended with styrene and a fluorescent 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) monomer, water-soluble and fluorescent core–shell nanoparticles (FNP) based on one-pot synthesis has been obtained in a miniemulsion RAFT polymerization, which are responsive to pH.[12] New molecules combining the functionalities of surface activity, polymerizability, and fluorescent properties within one molecule that could be seen as a fluorescent surfmer (surfactant and monomer) were successfully synthesized and utilized in one-pot production of fluorescent surface-labeled polymeric nanoparticles *via* miniemulsion polymerization.[13]

Schiff base compounds have attracted great research attention and been widely applied in the fields of medicine, catalysis, and analytical chemistry *et al*. With many advantages, such as mild reaction conditions and high reaction rates, Schiff base reaction was employed for protecting various functional groups and synthesizing a series of organic ligands.[14] Meanwhile, by

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combining some polymerization method, the Schiff base chemistry can provide an economic and convenient strategy to fabricate some functional polymers. An inexpensive, biocompatible self-healing hydrogel as a new injectable cell therapy carrier has been facilely developed through Schiff-base crosslinked chitosan by our group, that dynamic hydrogel could self-heal itself automatically without additional assistance.[15-16] Based on the work of Bentley and co-workers regarding reductive amination,[17-18] another strategy was developed by Haddleton's group to synthesize an aldehyde functionalized branched PEG polymers by ATRP and then conjugated to proteins by Schiff base reaction.[19-20] Furthermore, core-shell structure can also be achieved within a single molecule with a hyperbranched conjugated AIE polymer core and poly(ethylene glycol) arms through the acylhydrazone connection *via* Schiff-base chemistry.[21-22] Some aldehyde-functional polycarbonates (PCs) have been fabricated *via* ozonolysis and reductive work-up of allyl-functional PCs. The resulting aldehydes were functionalized with amino-oxy compounds under mild conditions through Schiff base linkages.[23] A novel double-hydrophilic block copolymer poly(2-(2'-methoxyethoxy)-ethyl methacrylate-*co*-oligo(ethyleneglycol) methacrylate)-*b*-poly(2-aminoethyl ethacrylate) (P(MEO_2MA-*co*-OEGMA)-*b*-PAMA) has been successfully synthesized with two-step RAFT polymerization. Then, the amino groups of PAMA blocks in the copolymers react with 1-pyrenecarboxaldehyde *via* the Schiff-base reaction, and the resulted copolymers are self-assembled to form spherical micelles in aqueous solution.[24]

In the last decade, fluorescent molecules and nano-objects have received increasing interest for their potential in biology and biochemistry, which are especially attractive for sensing, imaging and biomedical applications.[25] Owing to its good biocompatibility, low immunogenicity, and high water solubility, polyethylene glycol (PEG), a type of hydrophilic polymer, has been regarded as one of the best choices for surface modification of both inorganic and organic nanoparticles.[26] When a polymerizable AIE dye is employed into the polymerization of 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.[27-30] As a class of well-known AIE material, tetraphenylethene (TPE) derivatives have been widely developed for chemosensor and biomedical applications.[31-32] In this study, a novel one-pot strategy of smart combination of RAFT polymerization and *in situ* Schiff base reaction for fabrication of AIE-based copolymer was developed for the first time. The Schiff base reaction between amine donor monomer of N-(3-aminopropyl) methacrylamide (APMA) and AIE-based tetraphenylethene-benzaldehyde (TPEA) formed the target monomer TPEA-MA. Such obtained new monomers TPEA-MA synchronously participated in RAFT polymerization together with PEGMA to form a new amphiphilic copolymer with transformed fluorescent 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 copolymer were subsequently investigated.

2. Experimental

2.1. Materials and characterization

Poly(ethylene glycol) monomethacrylate (PEGMA, M_n = 500, J&K Chemical, AR), 2,2'-azobisisoheptonitrile (AVBN, J&K Chemical, 98%), N-(3-aminopropyl) methacrylamide (APMA, Tongchuang Pharma Co., Ltd, 98%), and triethylamine (TEA, J&K Chemical, AR) were all used as purchased. Tetraphenylethene-benzaldehyde (TPEA) and the chain transfer agent (CTA) of 4-cyano-4-(ethylthiocarbonothioylthio) pentanoic acid were prepared according to the literature methods.[33-34, 35]

Gel permeation chromatography (GPC) analysis of the copolymer was performed using N,N-dimethyl formamide (DMF) as the eluent. The GPC system was a Shimadzu LC-20AD pump system consisting of an auto injector, a MZ-Gel SDplus 10.0 mm guard column (50×8.0 mm, 10^2 Å) followed by an MZ-Gel SDplus 5.0 mm bead-size column (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}. ^{1}H NMR spectrum was obtained using a JEOL JNM-ECA 400 (400 MHz) 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 TPEA-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 TPEA-PEG suspension on a carbon-coated copper grid.

2.2. One-pot synthesis of fluorescent copolymer

The procedure for the one-pot synthesis of fluorescent copolymer TPEA-PEG was as follows: APMA (38 mg, 0.216 mmol), TPEA (94 mg, 0.216 mmol), PEGMA (323 mg, 0.646 mmol), TEA (30 mg, 0.290 mmol), CTA (2.8 mg, 0.01 mmol), AVBN (1.2 mg, 0.005 mmol) and 1.0 mL mixed solvent of acetonitrile/methanol/THF (v/v/v=1/1/1) were introduced into a Schlenk tube with a magnetic stir bar, and then followed by freeze-pump-thaw circle for three times. The Schlenk tube with reaction mixture was put into an oil bath maintained at 55 °C for 36 h. At the end of polymerization, the mixed solvent was removed with the rotary evaporator. The purified copolymer was obtained *via* precipitation from THF to petroleum ether for three times, and then dried under vacuum for further characterization (yield: 0.29 g). Finally, 10 mg TPEA-PEG copolymer was added to 5 mL H_2O, then shaken until it had been dissolved completely, which was used to investigated its self-assembly.

2.3. Cytotoxicity of TPEA-PEG FONs

The effects of TPEA-PEG FONs on A549 cells were examined by the observation of cell morphology.[36-37] In brief, cells were seeded into 6-well microplates in 2 mL of respective media containing 10% fetal bovine serum (FBS) at a density of 1×10^5 cells mL^{-1}. After cell attachment, plates were washed with PBS and cells were treated with complete cell culture medium, or different concentrations of TPEA-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. An optical microscopy (Leica, Germany) was used to observe the morphology of cells, whose overall magnification was ×100.

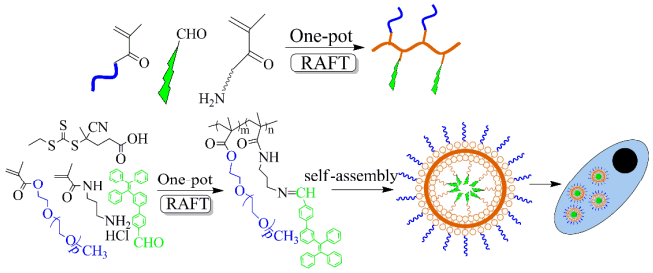
The cell viability of TPEA-PEG FONs on A549 cells was evaluated by cell counting kit-8 (CCK-8) assay on the basis of our previous reports.[38-39] In brief, cells were seeded in 96-well microplates with 160 μL of respective media containing 10% FBS at a density of 5×10^4 cells mL^{-1}. After cell attachment for 24 h, the cells were incubated with 10, 20, 40, 80, 120 μg mL^{-1} TPEA-PEG for 8 and 24 h. After nanoparticles were removed, the cells were then washed with PBS three times. 10 μL of CCK-8 dye and 100 μL of DMEM cell culture medium were added into each well and incubated for 2 h at 37 °C. Then the plates were analyzed with a microplate reader (Victor III, Perkin-Elmer). The absorbance of formazan dye was obtained at 450 nm, with the reference wavelength at 620 nm. The absorbance values were proportional to the number of live cells. The percent reduction of CCK-8 dye was compared to controls (cells not exposure to TPEA-PEG FONs), which represented 100% CCK-8 reduction. Per microplate experiment was repeated three times with three replicate wells. Cell survival was expressed as absorbance relative to that of untreated controls, and the results are presented as mean ± standard deviation (SD).

2.4. Confocal microscopic imaging (CLSM) of cells using TPEA-PEG FONs

The confocal microscopic imaging was used to evaluate the cell uptake of TPEA-PEG FONs.[15, 40] In brief, 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 TPEA-PEG FONs at a final concentration of 20 μg mL^{-1} for 3 h at 37 °C. Subsequently, to remove the TPEA-PEG FONs, the cells were washed three times with PBS and then fixed with 4% paraformaldehyde for 10 min at room temperature. Cell images were obtained with a confocal laser scanning microscope Zeiss 710 3-channels (Zeiss, Germany) with the excitation wavelength of 405 nm.

3. Results and discussion

In aqueous solution, the pH value of the solution significantly affects the reactivity of the Schiff base. Generally, higher acidity destroys the Schiff base, while the Schiff base becomes relatively stable in alkaline solution.[14] In this contribution, we reported a straightforward one-pot synthetic method through the combination of RAFT polymerization and *in situ* Schiff base monomer transformation. As Et_3N was added to keep the alkaline of solution, the target monomer (TPEA)-methacrylate (TPEA-MA) was facilely prepared by the Schiff base reaction between amino-containing monomer APMA and aldehyde-functionalized TPEA with excellent fluorescent. The as-prepared new monomers TPEA-MA together with PEGMA synchronously participated in RAFT polymerization to form a new copolymer with transformed fluorescent side groups. The introduction of TPEA and hydrophilic PEG would respectively endow the copolymer with fluorescence and excellent water solubility, thus the obtained water-soluble fluorescent copolymer was expected to self-assemble into nanoparticles and internalized by cell. The synthetic strategy in current report was schematically illustrated in Scheme 1.



Scheme 1 Schematic showing one-pot synthesis of TPEA-PEG copolymer through RAFT polymerization and Schiff base reaction, and then self-assembly of these copolymer for cell imaging.

The number average molecular weight (M_n) of the final obtained copolymer was about 29200 g mol^{-1} with a narrow PDI (~1.30, Fig. 1A). The ^1H NMR spectrum of the copolymer is also determined and shown in Fig. 1B. The characteristic peak of ester group of the PEGMA can be clearly observed at 4.05 ppm, and the aromatic hydrogen peak of TPEA appears clearly at the range of 7.12-7.87 ppm. The -N=CH- group in the Schiff base product is found to locate at 8.29 ppm, confirming the successful incorporation of TPEA into the copolymer. Depending on the integral ratio of the peaks at 8.29 ppm and 4.05 ppm, the molar fraction of TPEA and PEG in the copolymer can be calculated as approximate 20% and 80%, respectively.

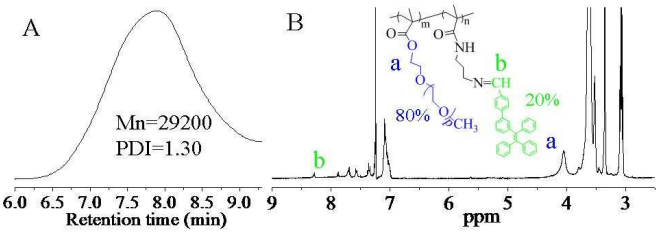


Fig. 1 (a) The GPC trace (DMF) and (B) ^1H NMR spectrum ($CDCl_3$) of the final obtained fluorescent copolymer.

The characterization information of the obtained TPEA-PEG FONs was further demonstrated in Fig. 2. Transmission electron microscopy (TEM) image of Fig. 2A shows that TPEA-PEG copolymer can self-assemble into uniform spherical nanoparticles with about 100 nm diameter. The self-assembly of the copolymer into spherical nanoparticles further confirmed that TPEA and PEGMA were successfully incorporated into the copolymer *via* Schiff base reaction and RAFT polymerization. Fig. 2B describes the UV absorption spectrum of TPEA-PEG FONs dispersed in water, and the entire spectrum starts to increase in absorption from 700 nm, indicating the formation of nano-aggregates in the solution.[41] 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. For the entire spectrum, the maximum absorption wavelength is located at 350 nm, which might contribute to the electron transition of π→π* because TPEA-PEG copolymer have the π-π conjugated structure. Owing to the surface of amphiphilic TPEA-PEG copolymer covered by hydrophilic PEG, they tended to self-assemble into nanoparticles in aqueous solution, forming PEGylated AIE-based FONs, so the obtained FONs could be well

dispersed in pure water solution. On the other hand, the TPEA-PEG FONs were endowed with the AIE property because the hydrophobic AIE dye (TPEA) in the core of FONs would partly aggregate in aqueous solution.[42] However, both TPEA-MA and PEGMA can dissolve in some organic solvents, making the obtained TPEA-PEG polymer have more excellent dissolution in organic solvent than in water. Fig. 2C shows the fluorescent difference between in water and in THF, the fluorescent property is clearly observed along with the maximum emission peak at 490 nm in the water solution, while in the THF solution, almost no fluorescence is observed, indicating 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.[43-44]

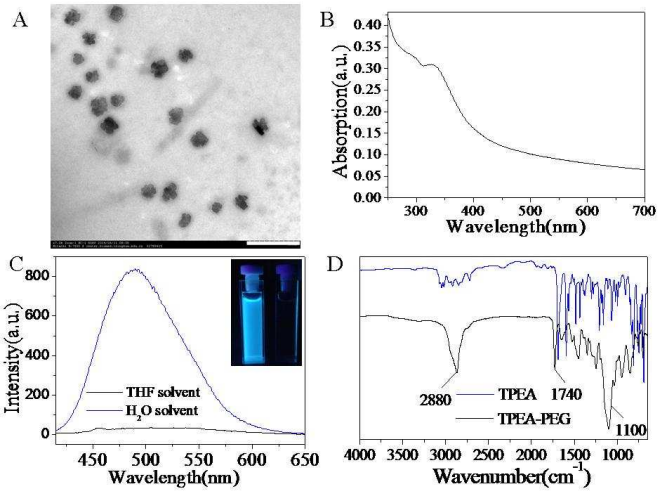


Fig. 2 Characterization of TPEA and TPEA-PEG FONs: (A) TEM image of TPEA-PEG FONs dispersed in water, scale bar=500 nm; (B) UV-Vis spectrum of TPEA-PEG dispersed in water; (C) Fluorescence emission spectra of TPEA-PEG FONs in water (left cuvette) and THF (right cuvette), inset is the fluorescent image of TPEA-PEG FONs taken at 365 nm UV light; (D) FT-IR spectra of TPEA and TPEA-PEG.

Fig. 2D demonstrates the FT-IR spectra of TPEA and TPEA-PEG copolymer. A series of absorbance bands of TPEA located between 1400 and 1600 cm^{-1} assigned to the stretching vibration of the polycyclic aromatic rings of TPEA can be observed. The C-H stretching vibration of polycyclic aromatic rings of TPEA presents at 2700~3100 cm^{-1}, moreover, two characteristic peaks of C=O and C=C located at 1710 and 1610 cm^{-1} can also be clearly observed. For the spectrum of TPEA-PEG, the C-H band of $-CH_2-$, $-CH_3$ and polycyclic aromatic rings located at 2880 cm^{-1} can also be clearly observed and the peak located at 1740 cm^{-1} is ascribed to the C=O stretching vibration. As compared with the spectrum of TPEA dye, the peak of TPEA-PEG copolymer located at 1710 cm^{-1} is almost disappeared, indicating the successful incorporation of TPEA into the copolymer by Schiff base reaction. On the other hand, the spectra of TPEA-PEG copolymer presents one characteristic peak of 1100 cm^{-1} (stretching vibration of C-O), further confirming the successful incorporation of PEGMA into the copolymer through RAFT polymerization. Combination of hydrophobic TPEA and hydrophilic PEG, the amphiphilic TPEA-PEG copolymer easily tends to self-assemble into uniform FONs in the water solution with the hydrophobic TPEA core covered by the hydrophilic PEG segments.

The biocompatibility of TPEA-PEG FONs was further evaluated by cell incubation with different concentrations of FONs for up to 24 h as shown in Fig. 3A-C. The optical microscopy observation results indicate that cells maintain their normal morphology. Even when the concentration of TPEA-PEG FONs is as high as 80 μg mL^{-1}, the cell morphology don't change clearly, implying the good biocompatibility of TPEA-PEG FONs. The good biocompatibility of TPEA-PEG FONs was further confirmed through A549 cell viability with cell counting kit-8 (CCK-8) assay as shown in Fig. 3D. The result demonstrates that the values of cell viability are more than 90%, when the cells are incubated with 10-120 μg mL^{-1} of TPEA-PEG FONs. Moreover, decrease of cell viability isn't clearly observed even when the concentration of TPEA-PEG FONs increases to 120 μg mL^{-1}. From the above results, it can conclude that TPEA-PEG FONs have good biocompatibility with cells and are promising for biomedical applications.

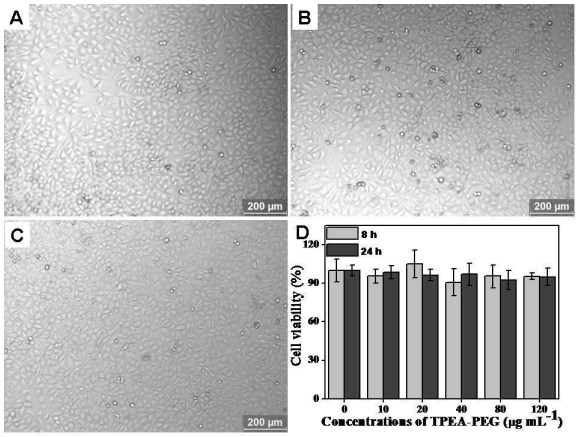


Fig. 3 Biocompatibility evaluations of TPEA-PEG FONs. (A–C) optical microscopy images of A549 cells incubated with different concentrations of TPEA-PEG FONs for 24 h, (A) control cells, (B) 20 μg mL^{-1}, (C) 80 μg mL^{-1}, (D) cell viability of TPEA-PEG FONs with A549 cells. The biocompatibility evaluation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

Due to the excellent water dispersibility, good fluorescent feature and favorable biocompatibility, the cell uptake behavior of TPEA-PEG FONs by CLSM was used to further investigate their potential cell imaging application as shown in Fig. 4A-C. After the cell was incubated with 20 μg mL^{-1} of TPEA-PEG FONs for 3 h, the strong fluorescence at the cytoplasm could be clearly observed, however, the areas with relatively weak fluorescence intensity should be the location of the cell nucleus (Fig. 4B).[45] These preliminary results suggest that TPEA-PEG FONs can be easily taken up by cells and mainly located at the cytoplasm. As compared with the size of FONs and nucleus pore, these FONs were considered to be taken up by endocytosis of the cells.[46] Based on the results of the cell viability, we believe that TPEA-PEG FONs combining the merits of AIE and PEG are biocompatible enough for bioimaging applications. More importantly, due to the controllability of RAFT polymerization and convenience of Schiff base reaction, novel monomers from Schiff base reaction can also be facilely integrated into polymer

chains, and thus multifunctional imaging and theranostic platforms can be obtained by controllable polymerization of AIE monomer from Schiff base reaction. In consideration of the excellent fluorescence and amphiphilic properties, these FONs should be promising for biomedical applications.

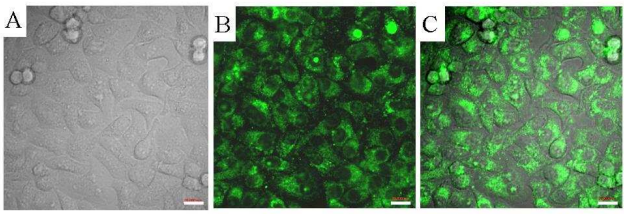


Fig. 4 CLSM images of A549 cells incubated with 20 μg mL^{-1} of TPEA-PEG FONs. (A) bright field, (B) excited with a 405 nm laser, (C) merged image of (A) and (B). Scale bar = 20 μm.

4. Conclusions

In conclusion, we have developed a novel one-pot strategy for the fabrication of TPEA-PEG FONs through controllable RAFT polymerization and Schiff base reaction of APMA and functional AIE dye (TPEA) for the first time. The M_n of as-prepared copolymer was about 29200 g mol^{-1} with a narrow PDI (~1.30), and the molar fraction of TPEA and PEG segments in the copolymer was about 20% and 80%, respectively. In water solution, these amphiphilic copolymer TPEA-PEG showed excellent water dispersibility owing to their surfaces covered with PEG and they tended to self-assemble into FONs, with the AIE dye as the hydrophobic core and PEG as the hydrophilic shell. Owing to the introduction of TPEA AIE dye, such FONs also showed excellent fluorescence in the water solution. TPEA-PEG FONs were biocompatible enough for bioimaging applications according to the biocompatibility evaluation. In view of the controllability of RAFT polymerization and the convenience of Schiff base reaction, many AIE based FONs can also be prepared through employing different AIE dyes. More importantly, various multifunctional FONs can also be easily fabricated for bioimaging applications by further conjugation with other components such as drugs, imaging agents, and targeting agents. In a word, the one-pot strategy combining RAFT polymerization and Schiff base technology provides an economical and efficient avenue for the preparation of numerous AIE-based FONs, which are promising for various biomedical applications.

Acknowledgements

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References

1 J. Geng, J. Lindqvist, G. Mantovani and D. Haddleton, *Angew. Chem., Int. Ed.*, 2008, 47, 4180-4183.
2 P. Golas and K. Matyjaszewski, *Chem. Soc. Rev*., 2010, 39, 1338-1354.
3 L. Mespouille, M. Vachaudez, F. Suriano, P. Gerbaux, O. Coulembier, P. Degée, R. Flammang and P. Dubois, *Macromol. Rapid Commun*., 2007, 28, 2151-2158.
4 B. van As, P. Thomassen, B. Kalra, R. Gross, E. Meijer, A. Palmans and A. Heise, *Macromolecules*, 2004, 37, 8973-8977.
5 F. Wolf, N. Friedemann and H. Frey, *Macromolecules*, 2009, 42, 5622-5628.
6 C. Fu, C. Zhu, S. Wang, H. Liu, Y. Zhang, H. Guo, L. Tao and Y. Wei, *Polym. Chem.*, 2013, 4, 264-267.
7 S. Wang, C. Fu, Y. Zhang, L. Tao, S. Li and Y. Wei, *ACS Macro. Lett.*, 2012, 1, 1224-1227.
8 Y. Zhang, C. Fu, C. Zhu, S. Wang, L. Tao and Y. Wei, *Polym. Chem.,* 2013, 4, 466-469.
9 C. Zhu, B. Yang, Y. Zhao, C. Fu, L. Tao and Y. Wei, *Polym. Chem.*, 2013, 4, 5395-5400.
10 Y. Wang, Y. Zhang, F. Zhang and W. Li, *J. Mater. Chem.*, 2011, 21, 6556-6562.
11 C. Shen, W. Yao and Y. Lu, *J. Nanopart. Res.*, 2013, 15, 2019-2033.
12 C. Grazon, J. Rieger, R. Méallet-Renault, G. Clavier and B. Charleux, *Macromol. Rapid Comm.*, 2011, 32, 699-705.
13 R. Sauer, A. Turshatov, S. Baluschev and K. Landfester, *Macromolecules*, 2012, 45, 3787-3796.
14 Y. Xin and J. Yuan, *Polym. Chem.*, 2012, 3, 3045-3055.
15 B. Yang, Y. Zhang, X. Zhang, L. Tao, S. Li and Y. Wei, *Polym. Chem.*, 2012, 3, 3235-3238.
16 Y. Zhang, L. Tao, S. Li and Y. Wei, *Biomacromolecules*, 2011, 12, 2894-2901.
17 M. Bentley, M. Roberts and J. Harris, *J. Pharm. Sci.*, 1998, 87, 1446-1449.
18 B. Droumaguet and J. Nicolas, *Polym. Chem.*, 2010, 1, 563-598.
19 L. Tao, G. Mantovani, F. Lecolley and D. Haddleton, *J. Am. Chem. Soc.*, 2004, 126, 13220-13221.
20 S. Ryan, X. Wang, G. Mantovani, C. Sayers, D. Haddleton and D. Brayden, *J. Control. Release*, 2009, 135, 51-59.
21 F. Qiu, C. Tu, R. Wang, L. Zhu, Y. Chen, G. Tong, B. Zhu, L. He, D. Yan and X. Zhu, *Chem. Commun.*, 2011, 47, 9678-9680.
22 R. Hu, N. Leung and B. Tang, *Chem. Soc. Rev.,* 2014, 43, 4494-4562.
23 G. Heo，S. Cho and K. Wooley, *Polym. Chem.*, 2014, 5, 3555-3558.
24 X. Dai, C. Hong and C. Pan, *Macromol. Chem. Phys.*, 2012, 213, 2192-2200.
25 A. Louie, *Chem. Rev.*, 2010, 110, 3146-3195.
26 X. Zhang, J. Hui, B. Yang, Y. Yang, D. Fan, M. Liu, L. Tao and Y. Wei, *Polym. Chem.*, 2013, 4, 4120-4125.
27 X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen and Y. Wei, *Polym. Chem.*, 2014, 5, 356-360.
28 X. Zhang, M. Liu, B. Yang, X. Zhang, Z. Chi, S. Liu, J. Xu, and Y. Wei, *Polym. Chem.*, 2013, 4, 5060-5064.
29 X. Zhang, X. Zhang, B. Yang, J. Hui, M. Liu, W. Liu, Y. Chen and Y. Wei, *Polym. Chem.*, 2014, 5, 689-693.
30 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.
31 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.
32 X. Zhang, Z. Chi, H. Li, B. Xu, X. Li, S. Liu, Y. Zhang and J. Xu, *J. Mater. Chem.*, 2011, 21, 1788-1796.
33 X. Zhang, Z. Chi, B. Xu, C. Chen, X. Zhou, Y. Zhang, S. Liu and J. Xu, *J. Mater. Chem.*, 2012, 22, 18505-18513.
34 X. Zhang, M. Liu, B. Yang, X. Zhang and Y. Wei, *Colloids and Surfaces B: Biointerfaces*, 2013, 112, 81-86.
35 L. Tao, J. Liu and T. Davis, *Biomacromolecules*, 2009, 10, 2847-2851.
36 X. Zhang, J. Yin, C. Peng, W. Hu, Z. Zhu, W. Li, C. Fan and Q. Huang, *Carbon*, 2011, 49, 986-995.
37 X. Zhang, M. Liu, B. Yang, X. Zhang and Y. Wei, *Colloids and Surfaces B: Biointerfaces*, 2013, 112, 81-86.

38 X. Zhang, H. Qi, S. Wang, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Toxicol. Res.*, 2012, 1, 201-205.
39 Z. Huang, X. Zhang, X. Zhang, C. Fu, K. Wang, J. Yuan, L. Tao and Y. Wei, *Polym. Chem.*, 2015, 6, 607-612.
40 X. Zhang, S. Wang, C. Fu, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Polym. Chem.*, 2012, 3, 2716-2719.
41 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.
42 X. Zhang, X. Zhang, L. Tao, Z. Chi, J. Xu and Y. Wei, *J. Mater. Chem. B*, 2014, 2, 4398-4414.
43 Y. Hong, J. Lam and B. Tang, *Chem. Commun.*, 2009, 4332-4353.
44 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.
45 X. Zhang, M. Liu, B. Yang, X. Zhang, Z. Chi, S. Liu, J. Xu, and Y. Wei, *Polym. Chem.*, 2013, 4, 5060-5064.
46 H. Li, X. Zhang, X. Zhang, B. Yang, Y. Yang and Y. Wei, *Polym. Chem.*, 2014, 5, 3758-3762.