Facile Synthesis of AIE-active Amphiphilic Polymers: Self Assembly and Biological Imaging Applications

Zi Long^{a,#}, Meiyong Liu^{a,#}, Ke Wang^b, Fengjie Deng^{a,*}, Dazhuang Xu^a, Liangji Liu^c, Yiqun Wan^a, Xiaoyong Zhang^{a,*}, Yen Wei^{b,*}

a Department of Chemistry, Nanchang University, 999 Xuefu Avenue, Nanchang 330031, China.

b Department of Chemistry and the Tsinghua Center for Frontier Polymer Research, Tsinghua University, Beijing, 100084, China.

c Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine, Nanchang, 330006, China

These authors contributed equally to this work

Abstract

In this work, we reported a rather facile method for fabrication of ultrabright, well dispersible and biocompatible fluorescent organic nanoparticles (FONs) with aggregation-induced emission (AIE) properties through combination of esterification and ring-opening reaction. The hydroxyl groups of Pluronic F127 was first reacted with the chloride of trimellitic anhydride chloride (TMAC), and its anhydride groups were further reacted with the amino groups of amino-terminated AIE dye (PhNH₂) through ring-opening reaction. The optical properties, biocompatibility as well as cell uptake behavior of these obtained AIE-active nanoparticles (F127-TMAC-PhNH₂ FONs) were examined by a series of characterization techniques and assays. We demonstrated that uniform organic nanoparticles with high water dispersibility, strong luminescence and desirable biocompatibility can be facily obtianed, which are promising for biological imaging applications. More importantly, a number of carboxyl groups were introduced into these AIE-active nanoparticles, which can be further utilized for further conjugation reaction and carrying anticancer drugs such as cisplatin. Therefore, the strategy of described in this work should be a simple and useful route for fabrication of multifunctional AIE-active luminescent nanotheranostic systems.

Key words: Ring-opening reaction, aggregation induced emission, biological imaging applications, drug delivery, Pluronic F127

1 Introduction

Fluorescent organic nanoparticles (FONs) have attracted increasing research attention because they play a significant role in biological imaging, bio/chemical sensing, light-emitting devices and disease diagnosis and treatment etc.[1-3] As compared with the fluorescent inorganic nanoparticles, FONs showed some attractive merits including low cost, superior brightness, biocompatibility and well designability of luminescent dyes and multifunctional potential.[4-18] The generally adopted methods for fabrication of FONs are conversion of hydrophobic dyes into amphiphilic polymers through direct encapsulation or different conjugation reactions.[19-21] These amphiphilic polymers will self assemble in pure aqueous solution and form core-shell organic nanoparticles, in which the hydrophobic dyes and other hydrophobic components were aggregated and served as the core, while the hydrophilic segments were coated on surface.[22-25] The self assembly procedure can in turn readily form high water dispersibility FONs for different biomedical applications. Unfortunately, it is still difficult to fabricate FONs with desirable luminescent properties using conventional organic dyes through self assembly procedure due to their notorious aggregation-caused quenching (ACQ) effect.[6] It is well known that the fluorescent intensity of conventional organic dyes will significantly decrease or completely quench at high concentration or solid state. The ACQ effect of convention organic dyes make great trouble for scientists to fabricate FONs because it is difficult to avoid the aggregation of dyes in pure aqueous solution during the self assembly procedure. Aggregation induced emission (AIE) is abnormal luminescence phenomenon, which was first observed and named by Tang et al in 2001.[26, 27] The most primary cause for AIE behavior is the restriction of intramolecular rotations (RIR) of peripheral phenyl (or heteroaryl) rotors in aggregated state. Therefore, AIE active dyes are expected to elegantly overcome the dilemma of ACQ and provide better choice for fabrication of ultrabright FONs through self assembly procedure.[28] Take advantage of AIE properties, a large number of AIE dyes such as tetraphenylethene, siloles, phenylethene, cyano-substituted diarylethene and distyrylanthracene derivatives have been synthesized and extensively examined for fabrication of ultrabright polymeric AIE probes. [29-33]

To date, multifarious AIE active FONs with intensive luminescence, higher water dispersibility, uniform size, and superb biocompatibility have been fabricated based on the strategies including direct encapsulation by amphiphilic polymers, covalent conjugation reactions, controlled radical living

polymerization, ring-opening reaction and formation of dynamic bonds.[34-39] These properties make AIE active polymeric nanoprobe promising candidates for bio-imaging applications *in vitro* and *in vivo*. [19, 40-42] Nevertheless, it is still a big challenge that such AIE dyes based self-assemble structures which are linear polymerization are usually unstable in dilute solution below the critical micelle concentration (CMC).[13] Furthermore, moderate synthesis strategies such as one-step synthesis, catalyst free, more versatile construction and eco-friendly performance are still highly desirable via using low cost and biocompatible commercial available amphiphilic polymers.

In this contribution, a rather neoteric undemanding method for preparing AIE active FONs was developed through combination of esterification reaction and ring-opening reaction. As shown in **Scheme 1**, a water soluble and biocompatible commercial available polymer (Pluronic F127) was first reacted with trimellitic anhydride acid chloride (TMAC) through a simple esterification reaction between hydroxyl groups and chloride groups of TMAC. Due to difference react activity between chloride groups and anhydride groups, the anhydride groups was preserved during the first step. It is worth to point out that the amino group of AIE dye (PhNH₂) can subsequently reacted with anhydride group of TMAC through ring-opening reaction. Thus obtained amphiphilic copolymers tend to self assemble into stable AIE active FONs (named as F127-TMAC-PhNH₂ FONs) with high water dispersibility. The size, morphology, composition and optical properties were characterized by a number of techniques in detail. To evaluate their potential biological imaging applications, the biocompatibility and cell uptake behavior of F127-TMAC-PhNH₂ FONs were further examined.

Scheme 1 Synthetic route showed the preparation of F127-TMAC-PhNH₂ FONs through combination of esterification and ring-opening reaction by taken advantage of the different reaction activity of TMAC toward hydroxyl and amino groups. The amphiphilic polymer Pluronic F127 was first conjugated with TMAC via esterification reaction to produce F127-TMAC. Then F127-TMAC was further reacted with amino-terminated AIE dye (PhNH₂) through ring-opening reaction to obtain F127-TMAC-PhNH₂ FONs.

2 Experiment

2.1 Materials and Characterization

Phenothiazine, sodium hydride, 1-Bromohexadecane, N,N-dimethylformamide, phosphoryl chloride, 4-aminobenzyl cyanide, tetrabutylammonium hydroxide (TBAH, 0.8 M in methanol), Pluronic F127 ($M_w = 12588$ Da), trimellitic anhydride chloride ($M_w = 210.57$ Da, 98%), dichloromethane and anhydrous THF were all purchased from Aladdin company (Shanghai, China), methylbenzene, dichloromethane, ethanol were purchased from Tianjin company, China. All chemicals were of analytical grade and were used as received without any further purification.

The synthetic polymers and materials were characterized by Fourier transform infrared spectroscopy (FT-IR) using KBr pellets. FT-IR spectra were supplied from Nicolet5700 (Thermo Nicolet corporation). ¹H nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance-400 spectrometer with D₂O and CDCl₃ as the solvent. 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 F127-TMAC-PhNH₂ ethanol suspension on a carbon-coated copper grid. The fluorescence data was obtained from the Fluorescence spectrophotometer (FSP, model: C11367-11), which purchased from Hamamatsu (Japanese). The hydrodynamic size distribution and Zeta potential of F127-TMAC-PhNH₂ FONs in water were determined using a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY). The CMC of F127-TMAC-PhNH₂ FONs was evaluated using fluorescence spectroscopy. The fluorescence intensity of F127-TMAC-PhNH₂ FONs with different concentrations was determined.

2.2 Preparation of PhNH₂

The synthesis of PhNH₂ and its intermediates is schematically shown in **Scheme 2**. Firstly, intermediate 1 was synthesized according to the literature.[43, 44] To obtain intermediate 2, phosphoryl

chloride (2.91 g, 19.0 mM) was slowly added to dry DMF (10 mL) at 0 °C, and the mixture was stirred for 2 h at room temperature. Then, a solution of intermediate 1 (4.28 g, 9.5 mM) in dichloromethane (30 mL) was added. After 1 h, the mixture was heated to 90 °C and stirred for 15 h. Finally, the solution was cooled and poured into 200 mL cool water and extracted with ethyl acetate. Drying with anhydrous magnesium sulfate, the solvent was removed and the residue was purified by column chromatography using a 1:1 mixture of dichloromethane and petroleum ether as an eluent to afford 2.61 g (58%) of compound 2 as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.87 (t, 3H), 1.17–1.35(m, 26H), 1.42 (quint, 2H), 1.80 (quint, 2H), 3.88 (m, 2H), 6.83–6.92 (m, 3H), 7.07–7.66 (m, 2H), 9.79 (s, 1H).

A solution of 2 (262 mg, 0.58 mM) and 2-(4-aminophenyl) acetonitrile (90 mg, 0.68 mM) in ethanol (10 mL) was stirred at room temperature. Then tetrabutyl ammonium hydroxide solution (0.8 M, 5 drops) was added and the mixture was heated to reflux for 12 h precipitating a red solid. The reaction mixture was cooled to room temperature, PhNH₂ (0.36 g, 91% yield). ¹H NMR (400 MHz, CDCl₃) δ (ppm): 0.87 (t, 3H), 1.18-1.31 (m, 26H), 1.36-1.68 (m, 2H), 1.79 (quint, 2H), 3.57-3.88 (s, 2H), 6.59-7.50 (m, 11H), 7.75 (s, 1H).

Scheme 2 Schematic showing synthesis route of PhNH₂ and its intermediates.

2.3 Fabrication of F127-TMAC-PhNH₂ FONs

The luminescent F127-TMAC-PhNH₂ FONs containing an AIE dye (PhNH₂) were prepared via fast esterification and ring-opening reaction according to a previous method.[45, 46] In brief, anhydride F127 (2.52 g, 0.2 mM) (In order to guarantee non-water in F127 samples, the water in purchased F127 was removed by azeotropic using toluene under vacuum rotary evaporation three times) were dissolved in 20 mL anhydrous THF solution in a dried flask. Next, TMAC (106 mg, 0.5 mM) in 10 mL dried THF solution was added dropwise into reaction system at room temperature (28 °C). Afterwards, 5

drops of TEA was added for reacting further 2 h. The white gelatinous precipitate F127-TMAC was gotten by rotary evaporation to remove the THF solution. And then F127-TMAC (1.29 g, 0.1 mM) and PhNH₂ (136 mg, 0.24 mM) were dissolved in 30 mL anhydrous THF solution. The ring-opening reaction was occurred via stirring the mixture under ambient atmosphere for 2 h. To purify, the resulting polymers should be frequently dissolved using ethyl acetate and participated using dry diethyl ether for three times. Finally, we got the orange solid F127-TMAC-PhNH₂ FONs.

2.4 Cytotoxicity of F127-TMAC-PhNH₂ FONs

The cell viability of F127-TMAC-PhNH₂ FONs on A549 cells was examined by cell counting kit-8 (CCK-8) assay.[47-50] Briefly, cells were seeded in 96-well microplates at a density of 5×10^4 cells per well in 160 μ L of respective media containing 10% fetal bovine serum (FBS). After 24 h of cell attachment, the cells were incubated with 20, 40, 60, 80 and 100 μ g mL⁻¹ of F127-TMAC-PhNH₂ FONs for 10 and 24 h, respectively. The F127-TMAC-PhNH₂ FONs were dispersed in the completely cell culture medium without using any organic solvents. Then nanoparticles were removed and the cells were washed with phosphate buffered solution (PBS) three times. 10 μ L of CCK-8 dye and 100 μ L of Dulbecco's modified eagle medium (DMEM) cell culture medium were added to each well and incubated for 2 h at 37 °C. The plates then were analyzed with a microplate reader (Victor III, Perkin-Elmer). Measurements of formazan dye absorbance were carried out at 450 nm, with a reference wavelength of 620 nm, as the values were proportional to the number of live cells. The percent reduction of CCK-8 dye was compared to control cells (no exposure to F127-TMAC-PhNH₂ FONs), which represented 100% CCK-8 reduction. Three replicate wells were used per microplate, and the experiment was repeated three times.

2.5 Laser Confocal microscopic imaging (CLSM) of F127-TMAC-PhNH₂ FONs

CLSM imaging of A549 cells using F127-TMAC-PhNH₂ FONs was conducted with a CLSM Zeiss 710 3-channel (Zeiss, Germany). The excitation wavelength was set as 458 nm. A549 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 maintained at 37 °C under a humidified condition of 95% air and 5 % CO₂ in culture medium. Culture medium was changed every three days to maintain the exponential growth of the cells. On the day prior to treatment, cells were seeded in a glass bottom dish with a density of 1×10^5 cells per dish. On the day of treatment, the cells were incubated with F127-TMAC-PhNH₂ FONs with a final concentration of 20 μ g mL⁻¹ for 3 h at 37 °C.

The F127-TMAC-PhNH₂ FONs were dispersed in the completely cell culture medium without using any organic solvents. Afterward, cells were washed three times with PBS to remove the F127-TMAC-PhNH₂ FONs and then fixed with 4 % paraformaldehyde for 10 min at room temperature.

3 Results and discussion

3.1 Characterization of F127-TMAC-PhNH₂ FONs

The chemical information of F127, F127-TMAC, PhNH₂ and F127-TMAC-PhNH₂ FONs was first evaluated by ¹H NMR spectroscopy. **Fig. S1** showed the ¹H NMR spectra of F127 and F127-TMAC in D₂O. As compared with the spectrum of F127, a novel peak with chemical shift at 6.6-7.8 ppm was emerged in the sample of F127-TMAC. The emerged signal can be attributed to the protons of aromatic rings in F127-TMAC, implying that TMAC has conjugated with the hydroxyl group of F127 through esterification reaction. More importantly, the anhydride groups were expected still retained on the end of F127-TMAC due to the low reaction activity of anhydride groups. On the other hand, the AIE dyes with amino groups can further conjugate with the anhydride groups through ring-opening reaction, which has been first developed by Zhang and Wei et al.[40] The ring-opening reaction has demonstrated to be one of the most effective methods for fabrication of AIE active polymeric nanoprobe due to its gentle reaction conditions. The successful formation of F127-TMAC-PhNH₂ FONs was also evidenced by ¹H NMR spectra. The ¹H NMR spectra of PhNH₂, F127-TMAC-PhNH₂ FONs in CDCl₃ were shown in **Fig. 1**. The protons of aromatic ring in PhNH₂ with chemical shifts around 7.5-8.3 ppm was observed in the sample of F127-TMAC-PhNH₂ FONs. On the other hand, the existence of a new chemical shift near 12 ppm was also confirmed by ¹H NMR spectra, which could be ascribed to signal of -COOH. Furthermore, the molar ratio between F127 and TMAC was also calculated based on ¹H NMR spectrum in **Fig. S1**. We demonstrated that ratio of F127 to TMAC is about 1:1.84. The results implied that almost all of the hydroxyl groups of F127 have reacted with chloride groups. All of the ¹H NMR spectra suggested that we have fabricated the polymeric AIE active nanoprobe through combination of esterification and ring-opening reactions.

Fig. 1 ^1H NMR spectra of PhNH_2 and F127-TMAC- PhNH_2 FONs. The solvent for ^1H NMR spectroscopy characterization is CDCl_3 .

The formation of F127-TMAC- PhNH_2 FONs was also confirmed by FT-IR. As compared with the FT-IR spectrum of F127, a new characteristic peak at 1700 cm^{-1} was observed in the samples of F127-TMAC, which can be attributed to the stretching vibration of the $\text{C}=\text{O}$ (**Fig. 2**). Furthermore, the $-\text{OH}$ peak around 3500 cm^{-1} in F127-TMAC has disappeared. The emergence of $\text{C}=\text{O}$ and disappearance of $-\text{OH}$ signals evidenced the successful formation of F127-TMAC. After PhNH_2 was linked with F127-TMAC via ring-opening reaction, a stretching vibration of $-\text{COOH}$ shown around 3400 cm^{-1} was observed in the samples of F127-TMAC- PhNH_2 FONs. On the other hand, two characteristic peaks at 1730 cm^{-1} and 1650 cm^{-1} could be considered as the stretching vibration of the $\text{C}=\text{O}$ of amide and carboxyl groups, respectively. The FT-IR spectra also confirmed formation of F127-TMAC- PhNH_2 FONs via combination of esterification and ring-opening reactions.

Fig. 2 FT-IR spectra of F127, F127-TMAC and F127-TMAC-PhNH₂ FONs samples, which provided evidence of successful formation of F127-TMAC-PhNH₂ FONs.

Due to their amphiphilic property, F127-TMAC-PhNH₂ copolymers were allowed to self-assemble into luminescent nanoparticles when they were dispersed in pure aqueous solution. In which, the hydrophobic AIE dye and PPO of F127 were aggregated and served as the core. However, the hydrophilic PEO segments of F127 and carboxyl groups were diffused into water, which was covered on the hydrophobic core as shell. Therefore, the obtained AIE dye contained copolymers will exhibit good water dispersibility and bright luminescence after self assembly. The excellent water dispersibility was also indicated by zeta potential measurement. We demonstrated that the zeta potential of F127-TMAC-PhNH₂ FONs is -39 mV. The negative surface charges could also indicated the good stability of F127-TMAC-PhNH₂ FONs. As expected, we found that F127-TMAC-PhNH₂ copolymers can be well dispersed in pure aqueous solution without any obvious agglomerates and precipitation (inset of left cuvette in **Fig. 3**). The water suspension of F127-TMAC-PhNH₂ was also examined using UV-Vis spectroscopy. As shown in **Fig. S2**, two adsorption peaks at 233 and 293 nm can be observed. The adsorption at 233 nm with strong intensity could be attributed to the amide II band of aromatic rings. And the adsorption at 293 nm with weak intensity could be attributed to the amide III band of aromatic rings. Therefore, we could conclude that PhNH₂ can conjugate with anhydride groups of

F127-TMAC through ring-opening reaction.

The fluorescent properties of F127-TMAC-PhNH₂ were further examined. The AIE properties of F127-TMAC-PhNH₂ can be observed in **Fig. S3**. When F127-TMAC-PhNH₂ copolymers were dispersed in the H₂O/THF mixture with 10% water, the F127-TMAC-PhNH₂ copolymers only emitted weak fluorescence. However, when F127-TMAC-PhNH₂ copolymers were dispersed in pure aqueous solution, the fluorescent intensity was obviously increased to about 5 folds. Furthermore, F127-TMAC-PhNH₂ copolymers can emitted strong and uniform yellow fluorescence in pure aqueous solution when they were irradiated by UV lamp with $\lambda = 365$ nm. The fluorescent images also suggested that the self assembly of F127-TMAC-PhNH₂ copolymers in aqueous solution. The PL spectra of F127-TMAC-PhNH₂ FONs were examined through fluorescent spectroscopy in detail. It can be seen that the emission peak of F127-TMAC-PhNH₂ FONs was located at 555 nm. And the maximum excitation peak of F127-TMAC-PhNH₂ FONs was located at 443 nm when the emission peak was set at 555 nm. More importantly, the emission peak location was almost no changes when F127-TMAC-PhNH₂ FONs were excited with different wavelength. On the other hand, the quantum yield (ΦF) of F127-TMAC-PhNH₂ FONs was calculated using Rhodamine 6G as the standard ($\Phi F = 95\%$).[51] We demonstrated that the ΦF of F127-TMAC-PhNH₂ FONs was as high as 11.6%. These results clearly demonstrated that F127-TMAC-PhNH₂ FONs possess excellent water dispersibility and PL properties. The size and morphology of the F127-TMAC-PhNH₂ FONs was characterized by TEM. As shown in **Fig. S4**, many uniform spherical particles with diameters of about 250-700 nm can be observed. Furthermore, the size distribution of F127-TMAC-PhNH₂ FONs based on TEM images was also analyzed. Results suggested that the size of F127-TMAC-PhNH₂ FONs is 465 ± 135.7 nm. The TEM characterization provided direct evidence for formation of F127-TMAC-PhNH₂ FONs. The formation of nanoparticles should be ascribed to the amphiphilic properties of F127-TMAC-PhNH₂ FONs. The self assembly procedure makes these F127-TMAC-PhNH₂ FONs good water dispersibility and high luminescence. The critical micelle concentration (CMC) of polymers is a very important parameter when they used as drug carriers or imaging probes. It is well known that the CMC of F127 is about 3 mg mL^{-1} . The chemical modification of F127 with other hydrophobic molecules has demonstrated to effectively reduce their CMC values. For example, Gao et al have demonstrated that the CMC of stearic acid-coupled F127 can as low as $0.69 \text{ } \mu\text{g mL}^{-1}$. [52] Therefore, the CMC of F127-TMAC-PhNH₂ FONs was determined based on our

previous studies.[53] In the work, we demonstrated that the CMC of F127-TMAC-PhNH₂ FONs is as low as 0.018 mg mL⁻¹. The low CMC of F127-TMAC-PhNH₂ FONs is very important for their biomedical applications as drug delivery carriers as well as luminescent probes.

Fig. 3 Fluorescence excitation and emission spectra of F127-TMAC-PhNH₂ FONs. The emission peak of F127-TMAC-PhNH₂ FONs was located at 555 nm, which is not changed using different excitation wavelength. And the maximum excitation peak is located at 443 nm when the emission peak wavelength was set at 555 nm. Insets are the images of F127-TMAC-PhNH₂ FONs water dispersion before and after irradiated by UV lamp ($\lambda = 365$ nm).

3.2 Biocompatibility of F127-TMAC-PhNH₂ FONs

To evaluate the potential biomedical applications of F127-TMAC-PhNH₂ FONs, their biocompatibility to A549 cells was also evaluated. In this work, the preliminary biocompatibility evaluation was conducted using CCK-8 assay, which has been previously adopted for estimating the effect of the biomaterials on the cell viability. The cell viability values of A549 cells after they were incubated with different concentrations of F127-TMAC-PhNH₂ FONs for both 10 and 24 h were displayed in **Fig. 4**. No significant cell viability value change was observed when the cells were incubated with 20-100 μ g mL⁻¹ F127-TMAC-PhNH₂ FONs for 10 h and 24 h. Even the concentrations of these FONs were as high as 100 μ g mL⁻¹, the cell viability value was unexpectedly greater than 100%, which is likely

ascribed to the cell uptake of F127-TMAC-PhNH₂ FONs will interference the CCK-8 assay measurement. One-way analysis of variance is used to evaluate the cell viability data. Results suggested that no significant difference was found between the controlled and experimental groups. These results affirmed that F127-TMAC-PhNH₂ FONs possess excellent biocompatibility and negative toxicity toward cells. As compared with other fluorescent inorganic nanoprobe and conventional organic nanoprobe, the AIE-active polymeric nanoprobe should be more suitable for biomedical applications for designability of surface functional groups, AIE properties in turn resulting bright luminescent intensity, excellent biocompatibility and potential biodegradability.

Fig. 4 The biocompatibility evaluation of A549 cells after incubating with different concentrations (20-100 µg mL⁻¹) of F127-TMAC-PhNH₂ FONs for 10 and 24 h.

3.3 Biological imaging of F127-TMAC-PhNH₂ FONs

The cell uptake behavior of F127-TMAC-PhNH₂ FONs was evaluated by CLSM observation. As shown in **Fig. 5**, strong orange fluorescence signal can be observed in cells after they were only incubated with 20 µg mL⁻¹ of these FONs. The fluorescent signal was overlapped on the cell location. And the areas with relative weaker fluorescent signal were surrounded by the areas with stronger fluorescent signal (**Fig. 5A**). We therefore conclude that F127-TMAC-PhNH₂ FONs could enter into cells and mainly locate at cytoplasm. Because no targeting agents were functionalized on the surface of

F127-TMAC-PhNH₂ FONs, the uptake of these FONs should be caused by endocytosis route. On the other hand, the cell uptake of F127-TMAC-PhNH₂ FONs could be further enhanced after their surface was conjugated with targeting agents. Thus, we could expect that the F127-TMAC-PhNH₂ FONs should be promising candidates for cell imaging and drug delivery applications with combined advantages, such as stable morphology in dilute physiological solution, strong fluorescence, good water dispersibility, and excellent biocompatibility. The cell uptake mechanism of AIE active nanoparticles has previously determined by Liu et al. In their work, the authors demonstrated that the cell uptake of folic acid modified AIE active nanoparticles can be influenced by cell culture temperature and folic acid in the cell culture medium. The internalization of these AIE active nanoprobe is through an energy dependent and receptor-mediated endocytosis route.[54] Because no targeting agents were immobilized on F127-TMAC-PhNH₂ FONs, therefore, F127-TMAC-PhNH₂ FONs are likely due to the nonspecific cell endocytosis route.

The preparation and their biomedical applications of AIE active polymeric nanoprobe have been extensively investigated in recent years. And a variety of AIE dyes with different chemical structure and functional groups were synthesized. These AIE-active dyes can be integrated into polymers to form dyes contained luminescent polymeric nanoprobe through different strategies.[39, 55, 56] For example, our previous reports have demonstrated the hydrophobic AIE dyes can be encapsulated into polymeric nanoprobe through surfactant assistant self assembly, controlled polymerization, ring-opening reaction and formation of dynamic bonds etc.[34-39] In this work, the water dispersible and biocompatible AIE-active polymeric nanoprobe have been fabricated using a novel covalent strategy taken advantage of the different reaction activity of TMAC toward hydroxyl group and amino groups. As compared with previous reports, this method is rather simple and effective. More importantly, many carboxyl groups were generated during the ring-opening reaction, which could not only be used for conjugation with the hydrophobic AIE dyes and polymers, but also provide the functional groups for further conjugation reaction and used for carrying drugs. Therefore, this method should be of great interest for fabrication AIE dyes based multifunctional nanotheranostic systems.

Fig. 5 CLSM images of A549 cells incubated with $20\text{ }\mu\text{g mL}^{-1}$ of F127-TMAC-PhNH₂ FONs for 3 h. (A) Bright field, (B) fluorescence images after excitation with a 458 nm laser, (C) merged image. Scale bar = 20 μm .

4. Conclusions

In conclusion, we have developed a novel strategy for the fabrication of AIE active FONs with superb biocompatibility, exceptional water dispersibility and fluorescence properties through a rather facile method via combination of AIE dye (PhNH₂) and F127. Take advantage of different reaction activity of TMAC toward F127 and PhNH₂, this strategy can ideally conjugate the hydroxyl groups of F127 and the amino groups of PhNH₂. Besides, plenty of carboxyl groups were present on F127-TMAC-PhNH₂ FONs; thus, many other functional components such as drugs, targeting agents and other imaging agents can be further integrated into F127-TMAC-PhNH₂ FONs. Therefore, multifunctional theranostic platforms based on F127-TMAC-PhNH₂ FONs can be fabricated. Combination all these advantages of F127-TMAC-PhNH₂ FONs, the orange AIE dye based FONs are deemed to be promising biomaterials for various biomedical applications.

Acknowledgements

This research was supported by the National Science Foundation of China (Nos. 21134004, 21201108, 51363016, 21474057, 21564006, 21561022, 81260537, 81460708), and the National 973 Project (Nos. 2011CB935700).

References

- [1] D. Ding, K. Li, B. Liu, B.Z. Tang, *Acc. Chem. Res.*, 46 (2013) 2441-2453.
- [2] R. Hu, N.L. Leung, B.Z. Tang, *Chem. Soc. Rev.*, 43 (2014) 494-4562.
- [3] X. Zhang, K. Wang, M. Liu, X. Zhang, L. Tao, Y. Chen, Y. Wei, *Nanoscale*, 7 (2015) 11486-11508.
- [4] C.W.T. Leung, Y. Hong, S. Chen, E. Zhao, J.W. Lam, B.Z. Tang, *J. Am. Chem. Soc.*, 135 (2013) 62-65.
- [5] H. Shi, R.T. Kwok, J. Liu, B. Xing, B.Z. Tang, B. Liu, *J. Am. Chem. Soc.*, 134 (2012) 17972-17981.
- [6] H. Shi, J. Liu, J. Geng, B.Z. Tang, B. Liu, *J. Am. Chem. Soc.*, 134 (2012) 9569-9572.
- [7] Z. Wang, S. Chen, J.W. Lam, W. Qin, R.T. Kwok, N. Xie, Q. Hu, B.Z. Tang, *J. Am. Chem. Soc.*, 135 (2013)

8238-8245.

- [8] M. Liu, X. Zhang, B. Yang, F. Deng, Y. Yang, Z. Li, X. Zhang, Y. Wei, *Macromol. Biosci.*, 14 (2014) 1712-1718.
- [9] M. Liu, X. Zhang, B. Yang, L. Liu, F. Deng, X. Zhang, Y. Wei, *Macromol. Biosci.*, 14 (2014) 1260-1267.
- [10] C. Ma, Q. Ling, S. Xu, H. Zhu, G. Zhang, X. Zhou, Z. Chi, S. Liu, Y. Zhang, J. Xu, *Macromol Biosci.*, 14 (2014) 235-243.
- [11] M. Liu, J. Ji, X. Zhang, X. Zhang, B. Yang, F. Deng, Z. Li, K. Wang, Y. Yang, Y. Wei, *J. Mater. Chem. B*, 3 (2015) 3476-3482.
- [12] X. Zhang, S. Wang, L. Xu, Y. Ji, L. Feng, L. Tao, S. Li, Y. Wei, *Nanoscale*, 4 (2012) 5581-5584.
- [13] X. Zhang, X. Zhang, S. Wang, M. Liu, L. Tao, Y. Wei, *Nanoscale*, 5 (2013) 147-150.
- [14] M. Liu, X. Zhang, B. Yang, F. Deng, J. Ji, Y. Yang, Z. Huang, X. Zhang, Y. Wei, *RSC Adv.*, 4 (2014) 22294-22298.
- [15] S. Chen, C. Zhang, G. Jia, J. Duan, S. Wang, J. Zhang, *Mater. Sci. Eng-C Mater.*, 43 (2014) 330-342.
- [16] V.N. Mehta, S. Jha, S.K. Kailasa, *Mater. Sci. Eng. C-Mater.*, 38 (2014) 20-27.
- [17] O. Kolmykov, J. Coulon, J. Lalevée, H. Alem, G. Medjahdi, R. Schneider, *Mat. Sci. Eng. C-Mater.*, 44 (2014) 17-23.
- [18] L. Ding, T. Li, Y. Zhong, C. Fan, J. Huang, *Mat. Sci. Eng. C-Mater.*, 35 (2014) 29-35.
- [19] W.-C. Wu, C.-Y. Chen, Y. Tian, S.-H. Jang, Y. Hong, Y. Liu, R. Hu, B.Z. Tang, Y.-T. Lee, C.-T. Chen, W.-C. Chen, A.K.-Y. Jen, *Adv. Funct. Mater.*, 20 (2010) 1413-1423.
- [20] K. Li, B. Liu, *Chem. Soc. Rev.*, 43 (2014) 6570-6597.
- [21] A.M. Breul, M.D. Hager, U.S. Schubert, *Chem. Soc. Rev.*, 42 (2013) 5366-5407.
- [22] H. Lu, X. Zhao, W. Tian, Q. Wang, J. Shi, *RSC Adv.*, 4 (2014) 18460-18466.
- [23] X. Zhang, M. Liu, B. Yang, X. Zhang, Z. Chi, S. Liu, J. Xu, Y. Wei, *Polym. Chem.*, 4 (2013) 5060-5064.
- [24] Z. Huang, X. Zhang, X. Zhang, C. Fu, K. Wang, J. Yuan, L. Tao, Y. Wei, *Polym. Chem.*, 6 (2015) 607-612.
- [25] Z. Huang, X. Zhang, X. Zhang, B. Yang, Y. Zhang, K. Wang, J. Yuan, L. Tao, Y. Wei, *Polym. Chem.*, 6 (2015) 2133-2138.
- [26] J. Luo, Z. Xie, J.W.Y. Lam, L. Cheng, H. Chen, C. Qiu, H.S. Kwok, X. Zhan, Y. Liu, D. Zhu, B.Z. Tang, *Chem. Commun.*, 37 (2001) 1740-1741.
- [27] J. Mei, N.L. Leung, R.T. Kwok, J.W. Lam, B.Z. Tang, *Chem. Rev.*, 115 (2015) 11718-11940.
- [28] M. Liu, X. Zhang, B. Yang, F. Deng, Z. Huang, Y. Yang, Z. Li, X. Zhang, Y. Wei, *RSC Adv.*, 4 (2014) 35137-35143.
- [29] Z. Chi, X. Zhang, B. Xu, X. Zhou, C. Ma, Y. Zhang, S. Liu, J. Xu, *Chem. Soc. Rev.*, 48 (2012) 3878-3896.
- [30] X. Zhang, X. Zhang, L. tao, Z. Chi, J. Xu, Y. Wei, *J. Mater. Chem. B*, 2 (2014) 4398-4414.
- [31] Y. Hong, J.W.Y. Lam, B.Z. Tang, *Chem. Soc. Rev.*, 40 (2011) 5361-5388.
- [32] L. Yan, Y. Zhang, B. Xu, W. Tian, *Nanoscale*, DOI: 10.1039/C1035NR05051K
- [33] Q. Lv, K. Wang, D. Xu, M. Liu, Q. Wan, H. Huang, S. Liang, X. Zhang, Y. Wei, *Macromol. Biosci.*, 16 (2016) 223-230.
- [34] X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen, Y. Wei, *Polym. Chem.*, 5 (2014) 356-360.
- [35] X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen, Y. Wei, *Polym. Chem.*, 5 (2014) 399-404.
- [36] X. Zhang, X. Zhang, B. Yang, J. Hui, M. Liu, W. Liu, Y. Chen, Y. Wei, *Polym. Chem.*, 5 (2014) 689-693.
- [37] X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen, Y. Wei, *Polym. Chem.*, 4 (2013) 4317-4321.
- [38] Q. Wan, K. Wang, C. He, M. Liu, G. Zeng, H. Huang, F. Deng, X. Zhang, Y. Wei, *Polym. Chem.*, 6

(2015) 8214-8221.

[39] Q. Wan, C. He, K. Wang, M. Liu, H. Huang, Q. Huang, F. Deng, X. Zhang, Y. Wei, *Tetrahedron*, 71 (2015) 8791-8797.

[40] Q. Wan, K. Wang, H. Du, H. Huang, M. Liu, F. Deng, Y. Dai, X. Zhang, Y. Wei, *Polym. Chem.*, 6 (2015) 5288-5294.

[41] K. Li, W. Qin, D. Ding, N. Tomczak, J. Geng, R. Liu, J. Liu, X. Zhang, H. Liu, B. Liu, *Sci. Rep.*, 3 (2013) 1150.

[42] K. Li, D. Ding, C. Prashant, W. Qin, C.-T. Yang, B.Z. Tang, B. Liu, *Adv. Healthcare Mater.*, 2 (2013) 1600-1605.

[43] X. Zhang, X. Zhang, B. Yang, L. Liu, J. Hui, M. Liu, Y. Chen, Y. Wei, *RSC Adv.*, 4 (2014) 10060-10066.

[44] X. Zhang, X. Zhang, S. Wang, M. Liu, Y. Zhang, L. Tao, Y. Wei, *ACS Appl. Mater. Interf.*, 5 (2013) 1943-1947.

[45] X. Zhang, X. Zhang, B. Yang, L. Liu, F. Deng, J. Hui, M. Liu, Y. Chen, Y. Wei, *RSC Adv.*, 4 (2014) 24189-24193.

[46] M. Liu, X. Zhang, B. Yang, F. Deng, Z. Li, J. Wei, X. Zhang, Y. Wei, *Appl. Surf. Sci.*, 322 (2014) 155-161.

[47] X. Zhang, W. Hu, J. Li, L. Tao, Y. Wei, *Toxicol. Res.*, 1 (2012) 62-68.

[48] X. Zhang, H. Qi, S. Wang, L. Feng, Y. Ji, L. Tao, S. Li, Y. Wei, *Toxicol. Res.*, 1 (2012) 201-205.

[49] X. Zhang, S. Wang, M. Liu, J. Hui, B. Yang, L. Tao, Y. Wei, *Toxicol. Res.*, 2 (2013) 335-346.

[50] Q. Wan, J. Tian, M. Liu, G. Zeng, Z. Li, K. Wang, Q. Zhang, F. Deng, X. Zhang, Y. Wei, *RSC Adv.*, 5 (2015) 25329-25336.

[51] X. Zhang, X. Zhang, B. Yang, Y. Zhang, M. Liu, W. Liu, Y. Chen, Y. Wei, *Colloids Surf. B Biointerfaces*, 1 (2014) 435-441.

[52] Q. Gao, Q. Liang, F. Yu, J. Xu, Q. Zhao, B. Sun, *Colloids Surf. B Biointerfaces*, 88 (2011) 741-748.

[53] H. Li, X. Zhang, X. Zhang, B. Yang, Y. Wei, *Colloids Surf. B Biointerfaces*, 121 (2014) 347-353.

[54] J. Geng, K. Li, D. Ding, X. Zhang, W. Qin, J. Liu, B.Z. Tang, B. Liu, *Small*, 8 (2012) 3655-3663.

[55] M. Liu, H. Huang, K. Wang, D. Xu, Q. Wan, J. Tian, Q. Huang, F. Deng, X. Zhang, Y. Wei, *Carbohydr. Polym.*, 142 (2016) 38-44.

[56] M. Liu, K. Wang, X. Zhang, X. Zhang, Z. Li, Q. Zhang, Z. Huang, Y. Wei, *Tetrahedron*, 71 (2015) 5452-5457.