RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

PAPER

Fabrication of amphiphilic fluorescent polylysine nanoparticles by atom transfer radical polymerization (ATRP) and their application in cell imaging

Zengfang Huang,*[ab] Xiqi Zhang,[bc] Xiaoyong Zhang,[b] Shiqi Wang,[b] Bin Yang,[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

Due to the good biocompatibility, Ɛ-polylysine (Ply) has been extensively investigated for various biomedical applications. In this study, a fluorescent monomer (named Flu-MA) was firstly synthesized through acylation reaction of fluorescein by methacryloyl chloride, and the initiator of Ɛ-polylysine bromide (named Ply-Br) was prepared by the introduction of bromine atom into Ply on the basis of acylation reaction of Ply by α-bromoisobutyryl bromide. Subsequently, a novel amphiphilic fluorescent polymer (Flu-Ply) was successfully fabricated by ATRP *via* incorporation of Flu-MA monomer into Ply chains for the first time. The structure and properties of the obtained Flu-Ply fluorescent polymer were investigated in detail by ^{1}H NMR, TEM, UV-vis, FL and FTIR, and the results confirmed the successful incorporation of Flu-MA into Ply by ATRP. As a result of Flu-MA and Ply respectively endowing the as-prepared Flu-Ply polymer with fluorescence and water dispersibility, they tended to self-assemble into fluorescent organic nanoparticles (FONs) with excellent biocompatibility. More importantly, the good fluorescence, uniform spherical morphology, excellent biocompatibility and water dispersibility of Flu-Ply FONs exhibited attractive prospect for bioimaging applications.

1. Introduction

With good biocompatibility, Ply is a naturally occurring food-grade antimicrobial that is produced by fermentation of the non-pathogenic and non-toxigenic microorganism *Streptomyces albulus*, which has been recognized as a safe substance in the scientific community and food preservative by FDA in October 2003.[1] For the chemical structure, Ply is a homopolymer of about 30 L-lysine monomers with a number of amino groups, which is linked together by isopeptide bonds between Ɛ-amino and α-carboxyl groups.[2-3] Ply has been extensively investigated for various biomedical applications ranging from enhancement of cell attachment to bioimaging, antimicrobial, gene delivery and nanocontainers, *et al*.[4-7] For example, using Ply as the linker, a novel method for the fabrication of cross-linked RO-OA(4,4'-oxydiphthalic anhydride)-Ply FONs has been developed through a one-pot RO (ring-opening) reaction.[8] Our group ever developed Ply-based AIE FONs through Schiff base condensation reaction, and the as-prepared FONs had uniform morphology , strong fluorescence, high aqueous dispersability, and excellent biocompatibility, being high potential for bioimaging.[1] Due to the limitation of Ply's tendency to associate with anionic substances, increasing product turbidity or forming sediments, a potential means of overcoming these problems was developed by forming electrostatic complexes between cationic Ply and anionic gum arabic (GA), and the result showed that the function of Ply cationic antimicrobial can be improved by incorporating it within electrostatic complexes using a food-grade GA anionic biopolymer,[9] which has important implications for the application of Ply in compositional complex systems.[10] The combination effects of Ply and nisin was also used to evaluate the synergistic action of these compounds against *Bacillus subtilis (B. subtilis)*, which indicated that the uptake of Ply into cells was promoted through nisin, subsequently Ply interacted with the intracellular DNA achieving a synergistic effect.[11] PEI-Ply multi-arm hyperbranched copolymers as gene carriers were successfully fabricated through the introduction of Ply to PEI (polyethylenimine) by RO polymerization of Ɛ-benzyloxycarbonyl-L-lysine N-carboxyanhydride, and the results revealed that PEI-Ply was a promising gene carrier for tumor gene therapy with the better tumor inhibition effects than those of PEI25k (hyper-branched PEI with a molecular weight of 25 kDa).[12] Hyperbranched amphiphilic polymeric systems can be used as versatile nanocontainers and templates with great potential in medicine application fields. The hydrophobic hyperbranched Ply was fabricated *via* polymer-analogue reaction using a mixture of stearoyl/palmitoyl chloride and glycidyl hexadecyl ether, respectively, which induced the formation of

[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*
[c]*Laboratory of Bio-Inspired Smart Interface Science, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing, 100190, P. R. China.*

Ag, Au, and Pd nanoparticles and nanocrystals in organic solvents and stabilized them.[13]

ATRP is a controllable polymerization method with organic bromide as initiator and transition metal complexes as carrier of bromine atom, and its controlling of polymerization reaction depends on the dynamic equilibrium of active species and dormant species. Otherwise, ATRP has the excellent tolerance to many monomers such as acrylate and styrene *et al.*, and can be used to fabricate gradient copolymer. ATRP has become one of the most widely used living radical polymerization technique for fabrication of polymers with pre-designed compositions, topologies and functionalities.[14-15] Our group prepared optically active polymer *via* 'one-pot' combination of transesterification reaction and ATRP, and the specific optical rotation [α] value of the obtained polymer was about -5.8° with narrow polydispersity indices (PDI～1.30).[16] The synthesis of fluorescent polymer *via* ATRP has attracted increasing attention. A novel functional monomer incorporating quinoline derivative moiety as the side group was synthesized, subsequently, the fluorescence polymer was prepared by ATRP, the fluorescence properties of which depended on both the monomer concentration in solution and the polarity of solvents.[17] A series of novel amphiphilic fluorescent CBABC-type pentablock copolymers were also synthesized by ATRP using CuBr/2,2-bipyridine as catalyst system, and the fluorescence spectra of the copolymers exhibited stronger excimer emission at *ca*. 480 nm due to the aggregations of pyrene group formed.[18] Otherwise, two compounds containing the benzothiazole moiety of BVMA and BPBVE were also synthesized, and the obtained homopolymer of PBVMA and copolymer of PBVMA-b-PMHNS by ATRP emitted blue fluorescence and orange fluorescence at about 610 nm due to the intramolecular energy transfer.[14] Fluorescent carbon nanoparticles (f-CNPs) have attracted a great deal of scientific attentions in recent years due to their high potential for biological and optoelectronic applications.[19-21] The f-CNPs were successfully grafted with polystyrene on the basis of a 'grafting from' method *via* ATRP. As compared with f-CNPs, the obtained f-CNP-g-PSt were fluorescent in solution or in the solid state and exhibit better dispersibility and processability.[22] In addition, from an in situ deactivation enhanced ATRP of multivinyl monomers (MVMs)，a new 3D single cyclized polymer chain structure was prepared, which are conventionally used for the production of branched/cross-linked polymeric materials.[23] Otherwise, from the controlled radical cross-linking copolymerization approach, the branched PDMAEMA copolymers were synthesized, which showed high potential for gene-delivery applications through a combination of the simplicity of their synthesis, their low toxicity, and their high performance. [24]

Recently, various FONs have been synthesized and extensively investigated for biomedical applications and many diagnostic assays.[25-27] As the food-grade and biocompatible polymer, Ply has been extensively investigated and applied in biology fields such as bioimaging, gene delivery and nanocontainers *et al*. As a class of xanthene compound, fluorescein is a very common fluorescence dye that is used for labeling purposes.[28-30] The synthesis methods of amphiphilic FONs mainly focused on RAFT and condensation polymerization for the bioimaging application, while it was rare for ATRP to be reported to fabricate amphiphilic FONs. In this contribution, a fluorescent monomer Flu-MA was synthesized through the acylation reaction of fluorescein by methacryloyl chloride, which was further incorporated into Ply polymer chains by ATRP with Ply-Br as initiator, affording novel fluorescent polymer with side fluorescent groups. Subsequently, the characterization results of ^{1}H NMR, TEM, UV-vis, FL and FTIR confirmed the successful incorporation of Flu-MA. In aqueous solution, the as-prepared amphiphilic Flu-Ply polymer tended to self-assemble into FONs. Finally, in order to investigate the cell bioimaging of Flu-Ply FONs, their biocompatibilities were further evaluated. As compared with the similar nanomicelles materials,[1,8] it was convenient and simple for the Flu-Ply polymer to be prepared, moreover, the obtained Flu-Ply polymer was sensitive to pH value of solution.

2. Experimental

2.1. Materials and characterization

Fluorescein (Flu, Aladdin agent, AR), N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA, Aladdin agent, 99%), methacryloyl chloride (MACl, Aladdin agent, 95%), α-bromoisobutyryl bromide (BiBB, Aladdin agent, 98%), copper bromide (CuBr, J&K Chemical, 98%) and triethylamine (TEA, J&K Chemical, AR) were all used as purchased.

^{1}H-NMR spectra were carried out on a JEOL JNM-ECA 400 (400 MHz) spectrometer at room temperature in a $CDCl_3$ and d_6-DMSO solution with tetramethylsilane (TMS) as a reference. Mass spectrum (MS) was performed with a TSQ Quantum Ultra mass analyzer (ThermoFisher). The transmission electron microscopy (TEM) specimen was made by placing a drop of Flu-Ply suspension on a carbon-coated copper grid, and TEM image was recorded on a JEM-1200EX microscope operated at 100 kV. UV-vis absorption spectrum of Flu-Ply FONs in water solution was performed on a Perkin-Elmer LAMBDA 35 UV-vis system. Fluorescence (FL) excitation and emission spectra of Flu-Ply FONs in water solution was measured on a PE LS-55 spectrometer. The FT-IR spectra of Flu-MA monomer and Flu-Ply polymer were obtained in a reflection mode on a Perkin-Elmer Spectrum 100 spectrometer (Waltham, MA, USA).

2.2. Synthesis of fluorescein methacryloyl (Flu-MA)

The synthesis of the fluorescent Flu-MA monomer was according to the literature method as follows:[29] Flu (3.0 g, 9.04 mmol) was dissolved in 20 mL CH_2Cl_2 with the addition of 1.54 g Et_3N (15.2 mmol). The mixture was stirred and cooled in the icewater bath, and then 5 mL CH_2Cl_2 solution of 1.10 g MACl (10.6 mmol) was dropped slowly into the reaction bottle with constant pressure funnel in the N_2 atmosphere, and then reacted for 90 min at this temperature and was continually stirred at room temperature overnight. After evaporation under reduced pressure, the residue was purified by chromatography on a silica gel column with CH_2Cl_2/CH_3OH (100:0.5 by volume) as eluent to give Flu-MA (2.70 g, 75% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.01-8.02 (1H, d, J=6.40Hz), 7.59-7.66 (2H, m), 7.25 (2H, s), 7.06-7.08 (1H, d, J=8.00 Hz), 6.81 (2H, s), 6.73 (1H, s), 6.55-6.62 (2H, m), 6.37 (1H, s), 5.80 (1H, s), 2.06 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm): 169.90, 165.85, 152.38, 135.70, 130.00, 129.35, 128.68, 126.84, 125.65, 124.49, 117.79, 116.79, 110.59, 103.22, 18.22; TOF MS m/z=401.1, $[M+H^+]^+$, calc. for $C_{24}H_{17}O_6$=401.1.

2.3. Synthesis of Ɛ-polylysine (Ply)-initiator (Ply-Br)

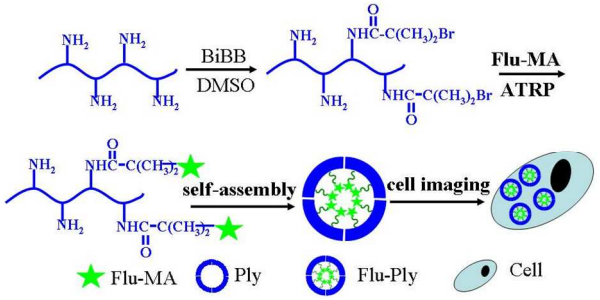
The synthesis of Ply-Br initiator was referring to the literature method as follows:[31] 5.0 g Ply was dissolved in 30 mL solvent of anhydrous dimethyl sulfoxide (DMSO) with the addition of 0.5 mL Et_3N, and then the 5 mL DMSO solution of 0.157 g BiBB (0.683 mmol) was dropped slowly into the reaction bottle with constant pressure funnel in the N_2 atmosphere. The above mixture was stirred at room temperature for 24 h, precipitated by the addition of THF, and then continually washed with THF three times. Finally, the product was dried under vacuum for the subsequent ATRP of Flu-MA monomer. Yield: 3.50 g.

2.4. Fabrication of amphiphilic fluorescent polymer by ATRP

The preparation of the fluorescent polymer Flu-Ply was as follows: Ply-Br (100 mg), Flu-MA (50 mg, 0.125 mmol), CuBr (15 mg, 0.106 mmol), PMDETA (34 mg, 0.197 mmol) and 3 mL DMSO solvent were added into a Schlenk tube consisting of a magnetic stir bar, and then followed by freeze-pump-thaw circle with nitrogen three times, subsequently, the above solution was dispersed by ultrasonic for 30 min. The reaction mixture was kept in an oil bath at 65 °C for 24 h, and then purified by dialysis with distilled water and methanol, respectively. Finally, the mixture was centrifuged at 9000 rpm for 30 min and dried under vacuum for further characterization.

3. Results and discussion

Herein, we reported the fabrication of amphiphilic fluorescent Flu-Ply by ATRP technique. Firstly, the fluorescent Flu-MA monomer was facilely prepared through acylation reaction of fluorescein by methacryloyl chloride with the addition of Et_3N to absorb the by-product HCl of the acylation reaction. The initiator of Ply-Br was accordingly fabricated by acylation reaction of Ply by BiBB in DMSO solvent. Subsequently, the new fluorescent polymer Flu-Ply was obtained through the introduction of Flu segment to Ply by the ATRP of Flu-MA taking Ply-Br as initiator in the presence of CuBr. The amphiphilic fluorescent Flu-Ply polymer with hydrophobic Flu-MA and hydrophilic Ply will be self-assembled into nanoparticles in aqueous solution and further internalized by cell. Scheme 1 illustrates the fabrication route of Flu-Ply FONs and their application for cell imaging.



Scheme 1. Schematic showing the fabrication of amphiphilic Flu-Ply FONs by ATRP technique and their self-assembly in aqueous solution for cell imaging.

The ^{1}H NMR spectra of Flu-MA monomer and Flu-Ply fluorescent polymer were described in Fig. 1. From the Flu-MA spectrum, the hydrogen peaks of $C=CH_2$ was clearly observed at 5.89 and 6.26 ppm with 1:1 integral area ratio, and the aromatic hydrogen peaks appeared obviously at the range of 6.56-8.00 ppm, moreover, the integral area ratio of 5.89, 6.26 and 6.56-8.00 ppm peaks was about 1:1:11, indicating that the acylation of fluorescein mainly produced the single acylation production as shown as in Fig. 1. For the Flu-Ply polymer, the characteristic aromatic hydrogen peaks of Flu-MA presented obviously at the range of 6.52-8.00 ppm, moreover, the $C=CH_2$ hydrogen peaks of Flu-MA at 5.89 and 6.26 ppm were disappeared, confirming the successful incorporation of Flu-MA into the Flu-Ply polymer chains by ATRP.

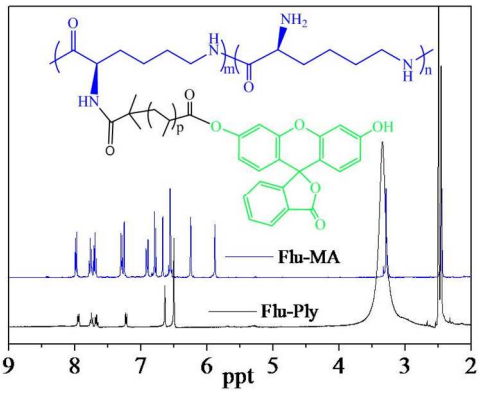


Fig. 1 ^{1}H NMR spectra (d_6-DMSO) of Flu-MA monomer and the final obtained Flu-Ply fluorescent polymer.

The characterization informations of the obtained Flu-Ply fluorescent polymer including TEM, UV-vis, FL and FTIR were detailedly described in Fig. 2. TEM image of Fig. 2A exhibited that Flu-Ply fluorescent polymer in aqueous solution had the sphere morphology with diameter of 100-200 nm, being consistent with the dynamic light scattering (DLS) result, which should be attributed to the self-assembly of Flu-Ply polymer in aqueous solution. The formation of sphere morphology also implied the successful incorporation of Flu-MA into the Flu-Ply polymer chains by ATRP. Fig. 2B described the UV-vis absorbance curve of Flu-Ply polymer in aqueous solution with the maximal absorbance at 480 nm. Because Flu-Ply polymer had the multi-phenyl structure with the linked hetero-atom O, the absorbance peak at 480 nm was possibly caused by the $n\rightarrow\pi^*$ electron transition of the phenyl ring. Due to the excellent water-solubility of Ply, the incorporation of hydrophobic Flu-MA into Ply polymer chains by ATRP would produce the amphiphilic Flu-Ply fluorescent polymer, which will self-assemble to form the corresponding Plyylated Flu-based FONs in water solution, so the obtained Flu-Ply FONs had high stability in aqueous solution. From the UV-vis spectrum of Flu-Ply, the curve was very flat without any absorption peak until the absorption wavelength decreased to 540 nm, indicating that the Flu-Ply solution had excellent aqueous dispersibility and wasn't affected by the light scattering or Mie effect which would decreased light transmission and caused the apparent high absorption and levelling-off of the tail in the visible region.[32] Due to the excellent fluorescence and dispersibility in water solution, the fluorescence excitation and emission spectra of Flu-Ply polymer in various pH value aqueous solution were described in Fig. 2C, and the spectra exhibited the maximal excitation wavelength at about 480 nm. Otherwise, the maximal fluorescence emission appeared at 520 nm, and its intensity increased with the pH value increasing. The result of the fluorescence intensity depending on pH value was possibly relevant to the tautomerizm structures of the fluorescent group in the Flu-Ply polymer side chain. In the various pH solution, the

Flu segment could exist as three molecule structures such as anion, neutral and cation structure, among which the anion and cation structure had respectively the highest and lowest quantum yield. For the Flu-Ply polymer, the Flu segment in polymer chains mainly existed as anion in alkaline medium but as cation in acidic solution, so the fluorescence intensity of the obtained Flu-Ply polymer was sensitive to pH value. Fig. 2D showed the FTIR spectra of Flu-MA monomer and its Flu-Ply polymer. From the Flu-MA spectrum, the absorption peak at 3350 cm^{-1} was found by the stretching vibration of -OH group, and the typical absorption peaks of C=O and C=CH_2 groups were observed at 1730 and 1620 cm^{-1} with a series absorption peaks of the polycyclic aromatic rings at the range of 1390-1560 cm^{-1}. For the Flu-Ply spectrum, the absorption peaks at the range of 3080-3270 cm^{-1} should be attributed to the stretching vibration of -OH group in Flu segment and -NH_2 group in Ply polymer. The absorption peak at 2930 cm^{-1} was caused by the stretching vibration of -CH_2- in Ply polymer. Otherwise, the absorption peaks at 1100 cm^{-1} were observed due to the C-N and C-O stretching vibration in Ply polymer and Flu segment. In a word, The FTIR spectrum of Flu-Ply polymer presented the composite structure of Flu-MA and Ply, which respectively endowed the Flu-Ply polymer with excellent fluorescence and high aqueous dispersibility, making them be possible for bioimaging applications.

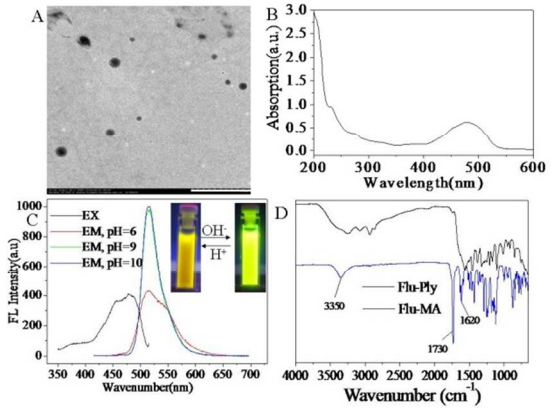


Fig. 2 Characterization informations of Flu-MA dye and Flu-Ply polymer: (A) TEM image of Flu-Ply FONs dispersed in aqueous solution, scale bar=1000 nm; (B) UV-vis spectrum of Flu-Ply dispersed in water; (C) Fluorescent excitation (EX) and emission (EM) spectra of Flu-Ply polymer in various pH value aqueous solution, inset is the fluorescence intensity variety of Flu-Ply polymer with the different pH value taken at 365 nm UV light; (D) FT-IR spectra of Flu-MA and Flu-Ply.

In order to study the bioimaging applications of the obtained Flu-Ply FONs, their biocompatibility with HepG2 cell was estimated by the cell morphology observation when they were incubated with various concentration of Flu-Ply FONs for 24 h as shown in Fig. 3,[33-36] and then the cell counting kit-8 (CCK-8) assay was further used to investigate the cell viability through the absorbance value of formazan dye at 450 nm taking 620 nm as the reference wavelength.[37-40] The optical microscopy observations indicated that no obvious change of cell morphology was observed with various concentrations of Flu-Ply FONs and the cells could still keep their normal morphology even when the concentration of Flu-Ply FONs increased to 80 μg mL^{-1} (Fig. 3A-C). Furthermore, the high biocompatibility of Flu-Ply FONs was also further confirmed through the cell viability study on the basis of the cell counting kit-8 (CCK-8) assay as shown in Fig. 3D.[41-43] The decrease of the cell viability wasn't obvious when the cell was incubated with the various concentration of Flu-Ply FONs, and the cell viability was still more than 90% even when cells were incubated with as high as 120 mg mL^{-1} of Flu-Ply FONs. From the above results, it can conclude that the obtained Flu-Ply FONs possess good biocompatibility and are promising for bioimaging applications.

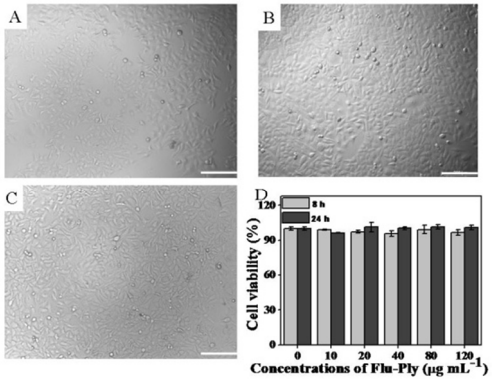


Fig. 3 Biocompatibility evaluations of Flu-Ply FONs. (A-C) optical microscopy images of HepG2 cells incubated with various concentrations of Flu-Ply FONs for 24 h, (A) control cells, (B) 20 μg mL^{-1}, (C) 80 μg mL^{-1}, (D) cell viability of Flu-Ply FONs with HepG2 cells. The biocompatibility evaluation indicated the good biocompatibility of Flu-Ply FONs with HepG2 and promising for biomedical applications. Scale bar = 200 μm.

Owing to the high aqueous dispersibility, good fluorescence and excellent biocompatibility of the Flu-Ply polymer, their uptake effect and cell imaging applications were evaluated by confocal laser scanning microscopy (CLSM) as shown in Figure 4 after the Flu-Ply FONs were uptaken by HepG2 cells.[44-46] The green dots were HepG2 cells, and the good cell uptake of Flu-Ply FONs was confirmed by the presence of the obvious green fluorescence. With careful observation, the Flu-Ply FONs were mainly located at the cytoplasm, and the core areas of the green dots with weak fluorescence should be the cell nuclei (Figure 4B).[47] Moreover, we consider that the cell uptake of nanoparticles was mainly due to their nano-size by the self-assembly in water solution together with the surface charge of the particles. From the above preliminary results, the obtained Flu-Ply FONs were considered to have high biocompatibility and could be applied in the bioimaging field. Finally, in consideration of the controllability of ATRP, it was possible for incorporating other polymerizable fluorescence monomers with different optical properties into various hydrophilic polymer by ATRP to fabricate FONs for bioimaging applications. Thus, it was anticipated to obtain multifunctional imaging and theranostic platforms by ATRP of polymerizable fluorescence monomers into hydrophilic polymer.

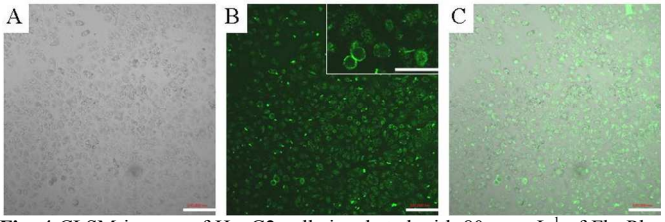


Fig. 4 CLSM images of HepG2 cells incubated with 80 μg mL^{-1} of Flu-Ply FONs. (A) bright field, (B) excited with a 488 nm laser, inset is the local zoom image, scale bar = 50 μm, (C) merged image of (A) and (B). Scale bar = 100 μm.

4. Conclusions

In summary, we prepared novel Flu-Ply FONs through controllable ATRP using hydrophilic Ply-Br as initiator and hydrophobic Flu-MA as polymerization monomer for the first time. The characterization informations of ^{1}H NMR, TEM, UV-vis, FL and FTIR confirmed that the fluorescent Flu-MA monomer was successfully incorporated into Ply. In aqueous solution, these amphiphilic Flu-Ply polymers exhibited excellent water dispersibility and tented to self-assemble into FONs with Flu-MA as hydrophobic core and Ply as hydrophilic shell. According to the biocompatibility evaluation, it was biocompatible enough for Flu-Ply FONs to be applied in bioimaging field. In consideration of the controllability and monomer applicability of ATRP, numerous FONs can also be facilely fabricated by ATRP through employing various fluorescent monomers and hydrophilic polymer. More importantly, by further conjugation with other components such as drugs, imaging agents, and targeting agents, *et al.*, many multifunctional FONs can also be facilely prepared for bioimaging applications. Taken together, ATRP provided an economical and efficient avenue for fabrication of multifunctional FONs, which was attractive for 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. X. Zhang, M. Liu, B. Yang, X. Zhang and Y. Wei, *Colloids and Surfaces B: Biointerfaces,* 2013, **112**, 81-86.
2. W. Nie, X. Yuan, J. Zhao, Y. Zhou and H. Bao, *Carbohyd. Polym.*, 2013, **96**, 342-348.
3. S. Shukla, A. Singh, A. Pandey and A. Mishra, *Biochem. Eng. J.*, 2012, **65**, 70-81.
4. I. Shih, Y. Van and M. Shen, *Mini Rev. Med. Chem.*, 2004, **4**, 179-188.
5. A. Artyukhin, O. Bakajin, P. Stroeve and A. Noy, *Langmuir,* 2004, **20**, 1442-1448.
6. D. Chattopadhyay, L. Galeska and F. Papadimitrakopoulos, *J. Am.Chem. Soc.*, 2003, **125**, 3370-3375.
7. D. Wang and L. Chen, *Nano. Lett.*, 2007, **7**, 1480-1484.
8. M. Liu, X. Zhang, B. Yang, L. Liu, F. Deng, X. Zhang and Y. Wei, *Macromol. Biosci.*, 2014, **14**, 1260-1267.
9. Y. Chang, L. McLandsborough and D. McClements, *Food Hydrocolloid.*, 2014, **35**,137-143.
10. Y. Chang, L. McLandsborough and D. McClements, *Food Res. Int.*, 2014, **64**, 396-401.
11. H. Liu, H. Pei, Z. Han, G. Feng and D. Li, *Food Control*, 2015, **47**, 444-450.
12. H. Tian, L. Lin, Z. Jiao, Z. Guo, J. Chen, S. Gao, X. Zhu and X. Chen, *J. Control Release*, 2013,**172**, 410-418.
13. C. Ho, M. Thiel, S. Celik, E. Odermatt, I. Berndt, R. Thomann and J. Tiller, *Polymer*, 2012, **53**, 4623-4630.
14. L. Zhang, Q. Xu, J. Lu, N. Li, F. Yan and L. Wang, *Polymer*, 2009, **50**, 4807-4812.
15. W. He, H. Jiang, L. Zhang, Z. Cheng and X. Zhu, *Polym. Chem.*, 2013, **4**, 2919-2938.
16. C. Fu, C. Zhu, S. Wang, H. Liu, Y. Zhang, H. Guo, L. Tao and Y. Wei, *Polym. Chem.*, 2013, **4**, 264-267.
17. Q. Xu, N. Li, F. Yan, X. Xia, J. Lu and X. Wen, *Eur. Polym. J.*, 2008, **44**, 1874-1880.
18. W. Chen, D. Liaw, K. Wang, K. Lee and J. Lai, *Polymer*, 2009, **50**, 5211-5219.
19. L. Shen, L. Zhang, M. Chen, X. Chen and J. Wang, *Carbon*, 2013, **55**, 343-349.
20. X. Guo, C. Wang, Z. Yu, L. Chen and S. Chen, *Chem. Commun.*, 2012, **48**, 2692-2694.
21. Y. Dong, C. Chen, J. Lin, N. Zhou, Y. Chi and G. Chen, *Carbon*, 2013, **56**, 12-17.
22. B. Liao, P. Long, B. He, S. Yi, Q. Liu and R. Wang, *Carbon*, 2014, **73**, 155-162.
23. Y. Zheng, H. Cao, B. Newland, Y. Dong, A. Pandit, and W. Wang, *J. Am. Chem. Soc.*, 2011, **133**, 13130-13137.
24. T. Zhao, H. Zhang, B. Newland, A. Aied, D. Zhou, and W. Wang, *Angew. Chem. Int. Ed.*, 2014, **53**, 6095-6100.
25. X. Zhang, X. Zhang, L. Tao, Z. Chi, J. Xu and Y. Wei, *J.Mater.Chem.B*, 2014, **2**, 4398-4414.
26. 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.
27. X. Zhang, Z. Chi, B. Xu, C. Chen, X. Zhou, Y. Zhang, S. Liu and J. Xu, *J. Mater. Chem.*, 2012, **22**, 18505-18513.
28. A. Breul, M. Hager and U. Schubert, *Chem. Soc. Rev.*, 2013, **42**, 5366-5407.
29. H. Wang, G. Zhou, H. Gai and X. Chen, *Chem. Commun.,* 2012, **48**, 8341-8343.
30. B. Bao, F. Li, H. Li, L. Chen, C. Ye, J. Zhou, J. Wang, Y. Song and L. Jiang, *J. Mater. Chem. C*, 2013, **1**, 3802-3807.
31. X. Zhang, C. Fu, L. Feng, Y. Ji, L. Tao, Q. Huang, S. Li and Y. Wei, *Polyme*r, 2012, **53**, 3178-3184.
32. 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.
33. X. Zhang, J. Yin, C. Peng, W. Hu, Z. Zhu, W. Li, C. Fan and Q. Huang, *Carbon*, 2011, **49**, 986-995.
34. X. Zhang, S. Wang, C. Zhu, M. Liu, Y. Ji, L. Feng, L. Tao and Y. Wei, *J. Colloid Interf. Sci.*, 2013, **397**, 39-44.
35. X. Zhang, X. Zhang, S. Wang, M. Liu, L. Tao and Y. Wei, *Nanoscale*, 2013, **5**, 147-150.
36. X. Zhang, W. Hu, J. Li, L. Tao and Y. Wei, *Toxicol. Res.*, 2012, **1**, 62-68.
37. X. Zhang, H. Qi, S. Wang, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Toxicol. Res*., 2012, **1**, 201-205.
38. X. Zhang, J. Hui, B. Yang, Y. Yang, D. Fan, M. Liu, L. Tao and Y. Wei, *Polym. Chem*., 2013, **4**, 4120-4125.
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. Z. Huang, X. Zhang, X. Zhang, B. Yang, Y. Zhang, K. Wang, J. Yuan, L. Tao and Y. Wei, *Polym. Chem.*, 2015, **6**, 2133-2138.
41. H. Qi, M. Liu, L. Xu, L. Feng, L. Tao, Y. Ji, X. Zhang and Y. Wei, *Toxicol. Res*., 2013, **2**, 427-433.
42. X. Zhang, S. Wang, M. Liu, J. Hui, B. Yang, L. Tao and Y. Wei, *Toxicol. Res*., 2013, **2**, 335-346.
43. K. Wang, X. Zhang, X. Zhang, B. Yang, Z. Li , Q. Zhang , Z. Huang and Y. Wei, *Macromol. Chem. Phys.*, 2015, **216**, 678-684.
44. X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen and Y. Wei, *Polym. Chem.*, 2014, **5**, 356-360.
45. B. Yang, Y. Zhang, X. Zhang, L. Tao, S. Li and Y. Wei, *Polym. Chem*., 2012, **3**, 3235-3238.
46. X. Zhang, S. Wang, C. Fu, L. Feng, Y. Ji, L. Tao, S. Li and Y. Wei, *Polym. Chem*., 2012, **3**, 2716-2719.
47. X. Zhang, M. Liu, B. Yang, X. Zhang, Z. Chi, S. Liu, J. Xu and Y. Wei, *Polym. Chem.*, 2013, **4**, 5060-5064.

Graphical abstract image

A novel amphiphilic Flu-Ply fluorescent polymer was successfully fabricated by ATRP method with the highly potential applications for bioimaging.