Synthesis, surface modification and biological imaging of aggregation-induced emission (AIE) dye doped silica nanoparticles

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Abstract

Fluorescent silica nanoparticles (FSNPs) have been extensively investigated for various biomedical applications in recently years. However, the aggregation of organic dyes in silica nanoparticles also leads the significant fluorescence quenching owing to the aggregation caused quenching effects of organic dyes. Herein, we developed a rather facile strategy to fabricate FSNPs with desirable fluorescent properties through non-covalent incorporation of fluorophores with aggregation-induced emission (AIE) feature into silica nanoparticles, which were subsequently modified with functional polymers. The resultant FSNPs polymer nanocomposites (named as FSNPs-poly(IA-co-PEGMA)) exhibited uniform spherical morphology, high water dispersiity, and bright red fluorescence. Cytotoxicity results indicate that FSNPs-poly(IA-co-PEGMA) possess excellent biocompatibility. Cell uptake behavior suggests FSNPs-poly(IA-co-PEGMA) are of great potential for biological imaging applications. Taken together, we have reported a facile method for the fabrication of FSNPs through non-covalent encapsulation using an AIE-active dye. These FSNPs can be further functionalized with functional polymers through ring-opening reaction and the resultant FSNPs-poly(IA-co-PEGMA) showed great potential for biological imaging. More importantly, we believe that many other functional components could also be integrated into these FSNPs through the facile ring-opening reaction. Therefore, this method should be a facile and general tool for fabrication of polymer functionalized AIE-active FSNPs.

Key words: Aggregation-induced emission, silica nanoparticles, surface modification, biological imaging

Introduction

Various inorganic nanomaterials, including Au nanoparticles[1], hydroxyapatite (HAp)[2], silica nanoparticles[3] or mesoporous silica nanoparticles (MSNs)[4, 5], play a pivotal role in the field of cell imaging and drug delivery. Owing to their unique physical and chemical properties, these materials could also be integrated with various fluorescent dyes or anti-cancer drugs for broadening their biological applications.[6-17] Among these hybrid systems, fluorescent silica nanoparticles (FSNPs) with quite fascinating properties have been extensively developed for biomedical applications. As compared with other fluorescent inorganic nanoparticles (FINs), FSNPs exhibited numerous advantages such as good optical transparency, controllable particle size, ease of surface functionalization and good cellular membrane-penetrating capacity.[18, 19] Such superior properties enable FSNPs better candidates for biomedical applications. However, FSNPs based on conventional organic dyes such as fluorescein and rhodamine etc have some limitations. It is noteworthy that these fluorescent dyes suffer from the obvious emission quenching, which is partially or completely because they experience intense intermolecular π - π stacking interactions.[20, 21] This phenomenon is known as aggregation-caused quenching (ACQ), which make the great challenge for fabrication of fluorescent nanoprobes with desirable optical properties because the aggregation of organic dyes is normally occurred in biological systems.

On the contrary, another type of fluorescent dyes with aggregation-induced emission (AIE) characteristics have attracted much research interest for fabrication of luminescent nanomaterials since the concept was coined by Tang group in 2001.[22-24] Compared with the conventional organic dyes, AIE-active dyes are weak fluorescence or non-fluorescence in dilute solutions whereas emit strong fluorescence in concentrated solutions or solids.[25] The mechanism of AIE phenomenon is interpreted as restriction of intramolecular rotations (RIR).[26] Thus it can be seen that AIE-active dyes are most suitable for fabrication multifunctional luminescent nanomaterials for biomedical applications (e.g. biological imaging or targeted therapy).[25, 27-41] Incorporation of AIE-active dyes into silica nanoparticles through both covalent and non-covalent methods have demonstrated to form AIE dyes doped fluorescent silica nanoparticles.[42-44] However, the covalent methods usually require complicated experimental procedures and long reaction time to conjugate AIE-active dyes with silica precursors. These AIE-active dyes containing silica precursors can be further used for fabrication of AIE-active dyes doped silica nanoparticles. Other than the covalent method, non-covalent

encapsulation of **AIE-active** dyes into silica matrix for doping silica nanoparticles provide a new opportunity.[45-49] This physical coating of **AIE-active** dyes is simple and effective compared to **covalent methods**. On the other hand, encapsulation of **AIE-active** dyes by other inorganic materials such as hydroxyapatite, layered double hydroxides and metal organic frameworks have also demonstrated by some groups.[50-56] However, to the best of our knowledge, the surface modification of these AIE-active **dyes** doped inorganic nanocomposites has not been investigated thus far.

Surface modification of silica nanoparticles with silicate derivatives such as amine and vinyl for grafting of specific target molecules or non-specific hydrophilic polymers is important to enhance their biocompatibility and physicochemical stability. In recent years, the surface of silica nanoparticles covering suitable a polyethylene glycol (PEG)-containing polymer attracted great attention owing to the good hydrophilicity and excellent biocompatibility of PEG.[57] In addition, due to the versatile end group chemistry of the polymers coated silica nanoparticles, most anti-cancer drugs such as cis-platinum and folic acid could be introduced to open up a wide of promising applications. Combined the fluorescent signal of the AIE dyes doped FSNPs with the biological activity of the PEG-containing polymers **make multifunctional FSNPs become promising candidates** for the integration of diagnosis and treatment.

In this contribution, we successfully design and fabricate core-shell FSNPs with fluorescent molecules as the core and hydrophilic polymers as the shell. As shown in **Scheme 1**, the **AIE-active dye 10-cetyl-10H-phenothiazine-3,7-(4,40-aminophenyl) acetonitrile (ATPHE)** as a contrast agent was first encapsulated in silica core via modified Stöber method. Then as-prepared AIE dyes doped FSNPs were modified with 3-aminopropyltriethoxysilane (APTES) to introduce amino groups for further surface modification. Finally, the functional polymers with anhydride group and PEG were immobilized on FSNPs through the ring-opening reaction. Due to the introduction of abundant carboxyl group and hydrophilic polymers, the functionalized FSNPs showed great water **dispersity** and functional groups for potential drug loading and delivery.

Scheme 1 Synthesis of FSNPs-poly(IA-co-PEGMA), (1) the AIE dye (ATPHE) encapsulated in the core of silica nanoparticles and surface modification of FSNPs with amino groups (FSNPs-NH₂), (2) the preparation of the poly(IA-co-PEGMA) via living free radical polymerization, (3) the fabrication of the polymers-coating FSNPs surface modified with the PEG-containing polymers through the ring-opening polymerization.

2. Experimental Section

2.1 Materials and characterization

Tetraethyl orthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTES), itaconic anhydride (IA), poly(ethylene glycol) methyl ether methacrylate (PEGMA, M_w : 950 Da, 98%), 2,2-azodiisobutyronitrile (AIBN) were purchased from Aladdin (Shanghai, China). Anhydrous toluene was obtained from Heowns (Tianjin, China). All other solvents were purchased from commercial sources and used directly without further purification. Deionized water was used in all experiments. The AIE dye, phenothiazine (named as ATPHE) containing two amino groups, was prepared according to our previous report.[58]

The fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Nicolet5700 (Thermo Nicolet corporation) using KBr pellets. In order to determine the element composition, the final products were characterized by X-ray photoelectron spectra (XPS), which was supplied from a VGESCALAB 220-IXL spectrometer. The morphology and size of the silica nanoparticles was measured using transmission electron microscopy (TEM) on a Hitachi 7650B microscope operated at 80 Kv, and the TEM specimens were made by placing a drop of the nanoparticles suspension on a

carbon-coated copper grid. The size distribution of FSNPs-poly(IA-co-PEGMA) in water was determined by dynamic laser scattering (DLS) based on a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY). Fluorescence spectra were measured on the fluorescence spectrophotometer (FSP, model: C11367-11, Japan).

2.2 Synthesis of FSNPs

The AIE-active dye (named as ATPHE) was synthesized according to our previous report and its chemical structure was listed in Fig. S1.[58] The obtained AIE dye (ATPHE) was encapsulated in silica NPs through a non-covalent embedment approach. First, ATPHE (0.1 mmol, 50 mg) was dissolved in 5 mL THF. Then 1 mL of the above solution was added to 16 mL ethanol, and 1.2 mL ammonium hydroxide was added. The reaction mixture was stirred at room temperature for 30 min. Subsequently, 0.5 mL TEOS and 1.5 mL ethanol were mixed and the mixture was added dropwise to the above reaction mixture, and then stirred for 20 h at room temperature. Finally, the resultant AIE dye containing silica nanoparticles were obtained by centrifugation (6000 rpm for 5 min) and were respectively washed three times with toluene and deionized water. The resulting products were dried in vacuum drying oven at 40 °C.

2.3 Synthesis of FSNPs-NH₂

The amine modified FSNPs were prepared via silane coupling reaction between the hydroxyl groups of the FSNPs and APTES. In brief, 200 mg FSNPs was dispersed in the solution with 60 mL ethanol and 20 mL water. Subsequently, 1.6 mL APTES was added to the above solution and stirred for 24 h at room temperature. The resulting amine modified FSNPs were separated by centrifugation (6000 rpm for 3 min) and were washed three times with ethanol and followed by dried in vacuum drying oven at 40 °C.

2.4 Synthesis of poly(IA-co-PEGMA)

The poly(IA-co-PEGMA) copolymers were synthesized via living free radical polymerization. In a typical procedure, PEGMA (1.8 g, 2 mmol), IA (450 mg, 4 mmol), AIBN (8 mg) and distilled dry THF (5 mL) were added in a 25 mL Schlenk tube. The tube was evacuated under vacuum and filled with dry N₂ three times prior to transfer to a preheated oil bath maintained at 70 °C for 10 h. After that, the poly(IA-co-PEGMA) was obtained via precipitation from THF to diethyl ether for three times. Finally, white precipitate was collected by filtration and drying.

2.5 Synthesis of FSNPs-poly(IA-co-PEGMA)

Preparation of FSNPs-poly(IA-co-PEGMA) was via ring-open reaction between the amine groups on the surface of the FSNPs-NH₂ and anhydride of poly(IA-co-PEGMA). The experimental procedure could be described as follows: poly(IA-co-PEGMA) (100 mg) and FSNPs-NH₂ (50 mg) were dispersed in anhydrous toluene. The mixture was stirred at 40 °C for 6 h. The synthesized FSNPs-poly(IA-co-PEGMA) were collected by centrifugation at 6000 rpm for 3 min and were washed at least three times with ethanol. The final products were dried in vacuum drying oven at 40 °C.

2.6 Cytotoxicity assay of FSNPs-poly(IA-co-PEGMA)

In order to evaluate the cytotoxicity of FSNPs-poly(IA-co-PEGMA), cytotoxicity assay was performed using cell counting kit-8 (CCK-8) assay.[59-62] In a typical procedure, cells were seeded in 96-well microplates at a density of 5×10^4 cells mL⁻¹ in 160 μL of respective media containing 10% fetal bovine serum (FBS). After 24 h of cell attachment, the cells were incubated with different concentrations of FSNPs-poly(IA-co-PEGMA) (20-160 μg mL⁻¹) for 12 and 24 h. Then FSNPs-poly(IA-co-PEGMA) were removed and cells were washed with phosphate buffer solution (PBS) three times. 10 μL of CCK-8 dye and 100 μL of DMEM cell culture medium were added to each well and incubated for 2 h at room temperature. Afterward, plates were analyzed using a microplate reader (VictorIII, Perkin-Elmer). Measurements of formazan dye absorbance were carried out at 450 nm, with the reference wavelength at 620 nm. The values were proportional to the number of live cells. The percent reduction of CCK-8 dye was compared to controls, which represented 100% CCK-8 reduction. Three replicate wells were used per microplate, and the experiment was operated for three times. Cell survival was expressed as absorbance relative to that of untreated controls. Results are presented as mean ± standard deviation (SD).

2.7 Confocal microscopic imaging

HeLa cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U mL⁻¹ penicillin, and 100 μg mL⁻¹ of streptomycin. Cell culture was controlled at 37 °C in a similar humidified condition of 95% air and 5% CO₂ in culture medium. Culture medium should be updated every three days for maintaining the exponential growth of the cells. Before 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 FSNPs-poly(IA-co-PEGMA) at a final concentration of 40 μg mL⁻¹ for 3 h at 37 °C. Afterward, the cells were washed three times with PBS to remove FSNPs-poly(IA-co-PEGMA) and then fixed with 4% paraformaldehyde for 10 min at room

temperature. Cell images were obtained using a confocal laser scanning microscope (CLSM) Zesis 710 3-channel (Zesis, Germany) with the excitation wavelength of 488 nm.

3. Results and discussion

The ATPHE with two amino groups was prepared according to our previous literature.[58] Its chemical structure is shown in **Fig. S1** and is characterized by standard spectroscopic techniques. It has been demonstrated that ATPHE containing into polymers copolymers show AIE-active feature. Therefore FSNPs-poly(IA-co-PEGMA) will also showed AIE characteristics owing to ATPHE was encapsulated in silica nanoparticles. As compared with our previous work, the size and morphology of silica nanoparticles can be well controlled. On the other hand, the surface of silica nanoparticles can be further coated with polymers and covered with mesoporous silica shells to endow novel functionalities (e.g. water dispersibility, high specific surface areas and functional groups), making these luminescent silica nanomaterials good candidates for fabrication of multifunctional theranostic systems. In this work, ATPHE as a fluorogen was non-covalently encapsulated into silica nanoparticles via a modified Stöber method. Then these as-prepared core-shell FSNPs were subsequently functionalized with APTES to introduce amino groups, which can be utilized for immobilization of the synthetic polymers poly(IA-co-PEGMA) through ring-opening reaction between amine and anhydride groups. The synthesized FSNPs-poly(IA-co-PEGMA) with bright fluorescence, controllable particle size, excellent biocompatibility and abundant carboxyl groups are promising for biomedical applications such as cell imaging and drug delivery.

The successful preparation of poly(IA-co-PEGMA) and FSNPs-poly(IA-co-PEGMA) were confirmed by ^1H NMR spectroscopy as a comparison. As shown in **Fig. 1**, the characteristic chemical shift ranging from 3.32 to 3.80 ppm is ascribed to the methylene groups of PEG and another characteristic chemical shift located in 4.26 ppm is attributed to the methylene groups of IA. The others peaks at $\delta = 0.82$ ppm ($-\text{CH}_3$), $\delta = 1.20$ ppm ($-\text{CH}_2-$), and $\delta = 1.94$ ppm ($\text{O}-\text{CH}_3$) are derived from the poly(IA-co-PEGMA). As compared with the ^1H NMR spectrum of poly(IA-co-PEGMA), the characteristic chemical shift of FSNPs-poly(IA-co-PEGMA) located in 3.61 ppm could be found because PEGMA could be dissolved in D_2O . Therefore, these results confirmed that poly(IA-co-PEGMA) have been successfully conjugated onto the surface of silica nanoparticles (FSNPs- NH_2) via ring-opening reaction.

Fig. 1 ^1H NMR spectra of poly(IA-co-PEGMA) and FSNPs-poly(IA-co-PEGMA).

The morphology and particle size of FSNPs could be clearly observed by the TEM images (**Fig. 2**). TEM image indicates that FSNPs possess a spherical shape and uniform particle size (about 110 nm). In addition, lots of obvious black spots could be clearly observed in the interior of FSNPs, which demonstrated that ATPHE had been successfully embedded into silica nanoparticles via a Stöber method. After surface modification of FSNPs-NH₂ with poly(IA-co-PEGMA) via ring-opening reaction between amine and anhydride groups, the size and morphology of silica nanoparticles was not obviously changed, however, many inter-particle linkage between silica nanoparticles were observed (**Fig. 2B**). Furthermore, the hydrodynamic size distribution of FSNPs-poly(IA-co-PEGMA) in water was determined by DLS. The results suggested that the size distribution of FSNPs-poly(IA-co-PEGMA) is 361 ± 8 nm, with a narrow polydispersity index (PDI) of 0.005 (**Fig S2**). The difference between TEM and DLS is possibly ascribed to the inter-particle linkage between silica nanoparticles. The above results implied that these silica nanoparticles were surface modification with polymer through the cross-linking reaction between amino groups of silica nanoparticles and anhydride groups of poly(IA-co-PEGMA).

Fig. 2 TEM images of (A) FSNPs, scale bar = 20 nm. (B) FSNPs-poly(IA-co-PEGMA), scale bar = 500 nm.

Fig. 3 illustrates the FT-IR spectra of FSNPs, FSNPs-NH₂ and FSNPs-poly(IA-co-PEGMA). As shown in **Fig. 3**, the absorption peak at 798 cm⁻¹ can be ascribed to the symmetric vibration of the Si-O groups, and the asymmetric vibration of the Si-O groups was located at 1095 cm⁻¹. Other characteristic absorption peak at 974 cm⁻¹ is ascribed to the asymmetric vibration of the Si-OH groups. There are three kinds of characteristic absorption bands can be clearly observed in the above three samples. In addition, the absorption peak of amino groups was appeared at 3468 cm⁻¹, evidencing the successful fabrication of FSNPs-NH₂ through silane coupling reaction between APTES and FSNPs. However, as compared with FSNPs and FSNPs-NH₂, the stretching vibration bands of C=O groups at 1653 cm⁻¹ was appeared in the simple of FSNPs-poly(IA-co-PEGMA), indicating that these FSNPs were surface functionalized with a dense polymer coating uccessfully.

Fig. 3 FT-IR spectra of FSNPs, FSNPs-NH₂ and FSNPs-poly(IA-co-PEGMA).

The chemical compositions of FSNPs, FSNPs-NH₂ and FSNPs-poly(IA-co-PEGMA) can be further confirmed by XPS. The survey scan spectra were shown in **Fig S3**. Five elements, i.e., silicon (Si), carbon (C), nitrogen (N), oxygen (O), and sulfur (S) were found in all the samples. It can also be observed that the major elements in these samples are Si, O and C. It is very interest to notice that intensive signals of C were observed in as-prepared FSNPs and FSNPs-NH₂. The C should originate

from the non-hydrolysis APTES and encapsulated AIE dye. Moreover, we could also clearly find that the signals of C in the sample of FSNPs-poly(IA-co-PEGMA) were obviously increased. This implied that poly(IA-co-PEGMA) polymers were introduced on FSNPs-NH₂ through ring-opening reaction between the amino groups of FSNPs-NH₂ and anhydride groups of poly(IA-co-PEGMA). The detailed elemental information of these samples can be further obtained from Si2p, C1s, N1s, O1s and S2p XPS spectra shown in Fig 4A-D and Fig S4. As compared with the sample of FSNPs, it is obvious that the N1s signal of FSNPs-NH₂ is significantly enhanced in N1s spectra, suggesting that APTES has been successfully immobilized on the surface of FSNPs through hydrolysis of APTES. In addition, it is clear to see that the peak intensity of Si2p continues to decline from the sample of FSNPs to FSNPs-poly(IA-co-PEGMA), meanwhile the peak intensity of C1s increased at first and then decreased. More importantly, there is a significant increase in O1s intensity of FSNPs-poly(IA-co-PEGMA) compared to FSNPs-NH₂. These results provide favorable evidence that poly(IA-co-PEGMA) has been immobilized onto the surface of FSNPs-NH₂ by ring-opening reaction. Unfortunately, the S2p spectra shown in Fig S4 are always very weak. The possible reason is that element S of ATPHE was encapsulated in the silica core and difficult to be detected by XPS.

Fig. 4 XPS spectra of FSNPs, FSNPs-NH₂ and FSNPs-poly(IA-co-PEGMA). (A) Si2p region, (B) C1s region, (C) N1s region and (D) O1s region.

Furthermore, **Table 1** shows the percentages of element content of FSNPs, FSNPs-NH₂ and FSNPs-poly(IA-co-PEGMA) based on XPS analysis. The pure FSNPs are mainly consisted of O (51.67%), Si (25.39%) and C (21.68%), and a little N (0.78%) and S (0.48%). Since FSNPs were modified with APTES, the percentages of N are increased from 0.78% to 2.80% and the percentages of other elements such as Si, C, O and S are changed to 19.36%, 38.76%, 38.43% and 0.65%, respectively. The increase of the percentages of N and C contents indicated that APTES has been successfully conjugated to the surface of FSNPs via hydrolysis reaction of APTES. Moreover, the percentages of O are increased from 38.43% to 59.16% after FSNPs-NH₂ was functionalized with poly(IA-co-PEGMA). Other element contents of Si, C, N and S are changed to 12.26%, 26.63%, 1.24% and 0.71%, respectively. Therefore, all the results suggested that poly(IA-co-PEGMA) have been coated on the surface of FSNPs-NH₂ via ring-opening reaction between amino groups of FSNPs-NH₂ and anhydride groups of poly(IA-co-PEGMA).

Table 1 Element contents of FSNPs, FSNPs-NH₂ and FSNPs-poly(IA-co-PEGMA) based on XPS analysis.

Sample	Si (%)	C (%)	N(%)	O (%)	S (%)
FSNPs	25.39	21.68	0.78	51.67	0.48
FSNPs-NH ₂	19.36	38.76	2.80	38.43	0.65
FSNPs-poly(IA-co-PEGMA)	12.26	26.63	1.24	59.16	0.71

Owing to encapsulation of **AIE-active** dye in silica nanoparticles, FSNPs-poly(IA-co-PEGMA) exhibit unique good water dispersity and intensive fluorescence. As shown in the inset of **Fig. 5**, uniform water suspension was found after FSNPs-poly(IA-co-PEGMA) deposited for over 12 h (left **cuvette**). On the other hand, bright and uniform red fluorescence could be obviously observed when FSNPs-poly(IA-co-PEGMA) water suspension was excited by UV lamp ($\lambda = 365$ nm). Furthermore, the fluorescence spectra of FSNPs-poly(IA-co-PEGMA) are shown in **Fig. 5**. **The maximum emission and excitation wavelength were centered at 595 and 495 nm, respectively. More importantly, we also found the excitation peak is very broad (from 390-595 nm). This implied that the FSNPs can be excited by the lasers with different wavelengths.** Moreover, it is worth to mention that as-prepared FSNPs-poly(IA-co-PEGMA) also exhibit excellent photo-stability. As shown in **Fig. S5**, only very few

fluorescent intensity decrease was found when FSNPs-poly(IA-*co*-PEGMA) were continuously irradiated by UV lamp at 365 nm for 1 h. Therefore, these results indicated that FSNPs-poly(IA-*co*-PEGMA) possess high water dispersity, unique optical properties and desirable photo-stability, suggesting their potential for biological imaging applications.

Fig. 5 (A) Fluorescence spectra of FSNPs-poly(IA-*co*-PEGMA) in distilled water. The inset images is the photographys of FSNPs-poly(IA-*co*-PEGMA) water suspensions; left (under natural light) and right (under UV lamp irradiation at 365 nm).

In order to further verify potential applications of FSNPs-poly(IA-*co*-PEGMA) in biomedical fields, the CCK-8 assay was carried out to evaluate cell viability of FSNPs-poly(IA-*co*-PEGMA). As shown in **Fig. 6**, it is obvious that no significant decrease of cell viability values could be observed after HeLa cells were incubated with 20-160 $\mu\text{g mL}^{-1}$ of FSNPs-poly(IA-*co*-PEGMA) for different time. More importantly, it is noteworthy that cell viability values are still greater than 90% even the concentrations of FSNPs-poly(IA-*co*-PEGMA) were arrived to 160 $\mu\text{g mL}^{-1}$. The results demonstrated that FSNPs-poly(IA-*co*-PEGMA) have excellent biocompatibility and low toxicity towards HeLa cells, indicating the promising potential for cell imaging application.

Fig. 6 Cell viability values estimated by CCK-8 assay using different concentrations of FSNPs-poly(IA-co-PEGMA). HeLa cells were incubated with FSNPs-poly(IA-co-PEGMA) for 12 and 24 h.

FSNPs-poly(IA-co-PEGMA) possess many remarkable properties, which include uniform particle size, unique optical properties, excellent biocompatibility and low toxicity. These features implied FSNPs-poly(IA-co-PEGMA) are suitable for biomedical applications. Therefore, the cell uptake behavior of FSNPs-poly(IA-co-PEGMA) was evaluated by CLSM observation. As shown in **Fig. 7A**, after HeLa cells incubated with $50 \mu\text{g mL}^{-1}$ of FSNPs-poly(IA-co-PEGMA) for 3 h. The cell plates were examined by CLSM after the cell plates were excited with 488 nm laser and intensive fluorescence signals were detected. The size of areas with fluorescent signals is about 20-30 μm . More importantly, many areas absent of fluorescence signals were surrounded by the areas with intensive fluorescent signals. Based on the above observation, we could conjecture that the areas with intensive fluorescence signals are the locations of cells while the areas in the center locations should be the positions of cell nuclei. The cell size, morphology and positions can be evidenced by the bright field image. As displayed from **Fig. 7B**, normal cellular morphology was maintained after cells were incubated with these luminescent nanoparticles for 3 h, further demonstrating the excellent biocompatibility of FSNPs-poly(IA-co-PEGMA). Moreover, the merged image shown in **Fig. 7C** clearly showed that the locations of cells were exactly overlapped by the fluorescence signals. Thus, the above results clearly confirmed that FSNPs-poly(IA-co-PEGMA) can be internalized into the cells through endocytosis and distributed mainly in the cell cytoplasm. Furthermore, as-prepared

FSNPs-poly(IA-co-PEGMA) are not only just suitable for cell imaging, but also possess a huge potential for many other biomedical applications. For example, a large number of carboxyl groups were generated during the procedure for immobilization of polymers on FSNPs. These carboxyl groups can be used for loading anticancer agent cisplatin and conjugating with targeting agents. It is therefore FSNPs-poly(IA-co-PEGMA) can be applied for drug delivery applications.

Fig.7 CLSM images of HeLa cells incubated with $40\text{ }\mu\text{g mL}^{-1}$ of FSNPs-poly(IA-co-PEGMA) for 3 h. (A) Fluorescent images were obtained by exciting with 488 nm laser, (B) Bright field, (C) merged image. Scale bar = $20\text{ }\mu\text{m}$.

4. Conclusion

In summary, we have developed a facile strategy to prepare core-shell FSNPs with the AIE-active dye ATPHE as the core and PEG-containing polymers as the shell. The obtained FSNPs-poly(IA-co-PEGMA) were characterized by TEM, FT-IR, XPS and PL spectroscopy. It can draw a conclusion that FSNPs-poly(IA-co-PEGMA) have been prepared successfully. The cell viability as well as cell uptake behavior has also been investigated to evaluate their potential for biomedical applications. Results suggested that FSNPs-poly(IA-co-PEGMA) exhibited low cytotoxicity and can be effectively internalized by HeLa cells. More importantly, due to the existence of abundant carboxyl groups on the surface of FSNPs-poly(IA-co-PEGMA). The FSNPs-poly(IA-co-PEGMA) can thus be further conjugated with many other functional components and utilized for carrying the anticancer agent. It is therefore multifunctional FSNPs can thus be fabricated and show great potential for various biomedical applications.

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Fluorescent silica nanoparticles with intensive luminescence, high water solubility and good biocompatibility were fabricated via direct encapsulation of aggregation-induced emission dye and subsequently surface modification of polymers through ring-opening reaction