Preparation of fluorescent organic nanoparticles from polyethylenimine and sucrose for cell imaging

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Abstract

An approach for the synthesis of fluorescent organic nanoparticles (FONs) with a quantum yield of about 14% has been developed using polyethylenimine and sucrose. The FONs were prepared under mild reaction condition. The obtained FONs showed high water dispersibility, intense fluorescence, excitation-dependent emission feature, excellent nanoparticle stability, and high photostability. The cytotoxicity of the FONs was also evaluated using A549 cells, and the cell viability value was demonstrated greater than 90% even when the concentration was up to 120 $\mu\text{g mL}^{-1}$, which proved excellent biocompatibility and made them promising for cell imaging.

Keywords: polyethylenimine; sucrose; fluorescent organic nanoparticles; biocompatibility; cell imaging

1. Introduction

Fluorescent organic nanoparticles (FONs) have generated extensive attention for cellular fluorescence imaging owing to their good biocompatibility, easy functionalization, and flexible synthetic approaches [1]. However, the organic dyes that form the FONs are prone to aggregate in water due to the poor miscibility, limiting their biological applications [2]. Moreover, aggregation of the organic dyes usually lead to fluorescence quenching as a result of strong intermolecular π - π interactions among the organic aromatic ring [3]. Another problem for traditional organic dyes is the photobleaching, also hampering their application as bioprobes [4]. Recently, aggregation induced emission (AIE) dyes have been introduced into the FONs. These AIE FONs have exhibited high quantum yield without fluorescence quenching, and rapidly aroused great interest of many researchers [5-7]. However, the preparation of AIE FONs require complex synthesized method and expensive reactant, limiting their large-scale synthesis. Fluorescent carbon dots (CDs), with low toxicity and good biocompatibility, have created immense attention in recent years and been widely used for bioimaging [8-10]. Various strategies for the preparation of fluorescent CDs have been reported, such as carbonizing organics, electrochemical oxidation, chemical oxidation and hydrothermal methods [11]. And some green synthetic approaches for the preparation of CDs have also been developed by using inexpensive renewable resources as precursors like honey [12], ginger [13], lychee seeds [14], and peanut shells [15]. However, most of these approaches suffer from either high reaction temperatures or long preparation times. In this case, preparation of fluorescent nanoparticles without complex synthesis, expensive reactant, high reaction temperatures, and long preparation times is highly demanded.

Polyethylenimine (PEI) is a polycationic polymer with repeating unit of amine and ethyl group. The highly reactive amino groups can react with epoxy, aldehyde, and isocyanate compound, and find many applications in formaldehyde adsorbent, dye fixing agent, and epoxy resin reaction agent. PEI is still a polymeric transfection agent and extremely attractive for building siRNA carriers [16-18]. Sucrose is a renewable, naturally occurring carbohydrate found in cane or beet sugar. The molecule is a disaccharide combination of the monosaccharides glucose and fructose, and hydrolysis will break the glycosidic bond converting sucrose into glucose and fructose.

In this study, a strategy of preparing FONs was developed for the first time *via* combining PEI and sucrose at mild condition (Scheme 1). The obtained FONs showed intense fluorescence, high water dispersibility, and excellent stability. The photostability and cytotoxicity of the FONs were evaluated,

and their cell imaging application has also been investigated.

2. Experimental procedure

2.1 Materials

Polyethyleneimine (PEI, $M_n = 600$) was purchased from Aladdin Industrial Corporation, sucrose was purchased from J&K Scientific Ltd. All other solvents and chemicals were of analytical grade and used without further purification.

2.2 Preparation of PEI-Suc FONs

A mixture of PEI (100 mg) and sucrose (200 mg) was dispersed in 20 mL deionized water, and then heated at 100 °C for 2 h. After cooling to room temperature, the mixture was purified by dialysis through porous cellulose bag (molecular weight cut off 3500 Da) using ethanol. Finally, the **PEI-Suc** FONs inside the dialysis bag was collected and dried by vacuum oven at 40 °C.

2.3 Characterizations

Transmission electron microscopy (TEM) image was obtained on a HT7700 microscope (Hitachi, Japan) operated at 100 kV, the TEM samples in solution phase were drop-cast onto a carbon-coated copper grid, and the grid was air dried at room temperature (25 °C) before loading into the microscope. Size distributions of **PEI-Suc** FONs in phosphate buffer solution (PBS) was measured using a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY). X-ray photoelectron spectra (XPS) were carried out on a VGESCALAB 220-IXL spectrometer equipped with an Al Ka X-ray source (1486.6 eV). The energy scale was internally calibrated by referencing to the binding energy (Eb) of the C1s peak of a carbon contaminant at 284.6 eV. Fourier transform infrared spectroscopy (FT-IR) spectra were conducted in a transmission mode on a Shimadzu Spectrum 8400 spectrometer (Japan). Typically, 8 scans at a resolution of 1 cm⁻¹ were accumulated to obtain one spectrum. For XPS and FT-IR measurement, the fluorescence dye was characterized in dried state. UV-Visible absorption spectrum was recorded on UV/Vis/NIR 2600 spectrometer (Shimadzu, Japan) using quartz cuvettes of 1 cm path length. Fluorescence spectra were measured on an F-4600 spectrometer with a slit width of 3 nm for both excitation and emission.

2.4 Cytotoxicity of PEI-Suc FONs

The cell viability of **PEI-Suc** FONs on A549 cells was evaluated by cell counting kit-8 (CCK-8) assay. Briefly, cells were seeded in 96-well microplates at a density of 5×10^4 cells mL⁻¹ in 160 µL of respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 10,

20, 40, 80, 120 $\mu\text{g mL}^{-1}$ **PEI-Suc** FONs for 8 and 24 h. Then nanoparticles were removed and cells were washed with PBS three times. 10 μL of CCK-8 dye and 100 μL of DMEM cell culture medium were added to each well and incubated for 2 h at 37 °C. Plates were then analyzed with a microplate reader (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 (cells not exposure to **PEI-Suc** FONs), which represented 100% CCK-8 reduction. Three replicate wells were used per microplate, and the experiment was repeated three times. Cell survival was expressed as absorbance relative to that of untreated controls. Results are presented as mean $\pm$ standard deviation (SD).

2.5 Confocal microscopic imaging of cells incubated with **PEI-Suc** FONs

A549 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 $\mu\text{g mL}^{-1}$ of streptomycin. Cell culture was maintained at 37 °C in a humidified condition of 95% air and 5% CO_2 in culture medium. Culture medium was changed every three days for maintaining 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 **PEI-Suc** FONs at a final concentration of 10 $\mu\text{g mL}^{-1}$ for 3 h at 37 °C. Afterward, the cells were washed three times with PBS to remove the **PEI-Suc** FONs and then fixed with 4% paraformaldehyde for 10 min at room temperature. Cell images were taken with a confocal laser scanning microscope (CLSM) Zesis 710 3-channel (Zesis, Germany) with the excitation wavelength of 405 nm and 458 nm.

3. Results and discussion

3.1 Characterization of **PEI-Suc** FONs

For the characterization of the morphology, chemical composition, and fluorescent properties of the **PEI-Suc** FONs, the following techniques, including transmission electron microscopy (TEM), dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), UV-Visible absorption spectrum, and fluorescence spectra were investigated to determine the properties of **PEI-Suc** FONs.

The morphology and structure of the **PEI-Suc** FONs were characterized by TEM (Fig. 1A). The prepared FONs are well dispersed and exhibited a spherical morphology with diameters ranging from 10 to 20 nm. The TEM images provided direct evidence of the formation of nanoparticles in aqueous

solution. Meanwhile, as shown in Fig. 1B, the size distribution of **PEI-Suc** FONs in phosphate buffer solution (PBS) was conducted on a zeta Plus particle size analyzer, indicating the size distribution at the range of 5-29 nm, with an average size of 17 nm. The size distribution of the FONs from DLS was in consistent with the TEM result.

The functional groups on the **PEI-Suc** FONs were investigated using FT-IR spectra. As shown in Fig. 2, in the sample of PEI, the characteristic peak located at 3275 cm^{-1} was assigned to the -NH_2 group, another two peaks at 2936 and 2817 cm^{-1} could be ascribed to the stretching vibrations of the -CH_2 groups. Moreover, the peaks at 1577 cm^{-1} and 1064 cm^{-1} assigned to bending vibration of -NH_2 and stretching vibration of the C-N bond were also found in PEI. For the sample of sucrose, the broad peak of -OH located at 3328 cm^{-1} could be corresponded to the stretching vibration of the -OH group, while strong stretching vibration of the C-O band was found at 1040 cm^{-1} . After reaction of PEI and sucrose at $100\text{ }^\circ\text{C}$ for 2 h to obtain the **PEI-Suc** FONs, several characteristic peaks could be clearly observed. The signal of -NH_2 and -OH groups was found at 3275 cm^{-1} , and the -CH_2 group located at 2982 and 2825 cm^{-1} was also observed, which was corresponded to that of PEI. Strong stretching vibrations of the C-O bond found at 1041 cm^{-1} and the C-N bond located at 1084 cm^{-1} also emerged in the FONs. More importantly, the stretching vibration of the C=N band located at 1630 cm^{-1} and bending vibration of $=\text{C-H}$ group located at 808 cm^{-1} could be clearly identified, demonstrating the reaction possibly involved hydrolysis of sucrose and formation of Schiff base between PEI and glucose.

The XPS spectra were also conducted to analysis the surface state and composition of the **PEI-Suc** FONs. As shown in Fig. 3A, three peaks at 285, 400 and 532 eV exist in the XPS spectra, indicating the presence of C, N and O elements in the **PEI-Suc** FONs, respectively. The overall wt % of elements present in the FONs was calculated as C:N:O ~ 66:13:21. The high resolution spectrum of C1s exhibits three main peaks located at 284.8, 286.2 and 288.6 eV, which are attributed to C-C, C-O and C=N, respectively (Fig. 3B). This result proved the formation of C=N bond in the Schiff base resulted from the condensation of PEI and glucose, as the hydrolysis of sucrose afforded the glucose, which was also confirmed by the FT-IR spectra. The high resolution spectrum of N 1s spectrum showed peak centered at 399.5 eV (Fig. 3C), while the high resolution spectrum of O 1s spectrum showed peaks at 531.8 eV (Fig. 3D). All these results supported that PEI and sucrose successfully generated the **PEI-Suc** FONs.

The inset of Fig. 4A showed that **PEI-Suc** FONs were readily dispersed in water with high

transparency. Then, the UV absorption spectrum of the as-prepared **PEI-Suc** FONs was investigated (Fig. 4A). The absorption spectrum showed a wide absorption band without any obvious characteristic peak, possibly due to the relatively broad size distribution of the FONs. Meanwhile, it is found that the absorption spectrum started to increase from 750 nm, indicating the existence of nanoparticles in the solution caused by the Mie effect [19]. The PL spectra of the **PEI-Suc** FONs were shown in Fig. 4B, which showed obvious excitation-dependent feature. The emission peaks shifted from 460 to 524 nm with an increase in the excitation wavelength ranging from 320 to 480 nm, which covered the wavelength ranging from blue to green. Moreover, the excitation-dependent feature of FONs have also been demonstrated by some other types of fluorescent nanoparticles [20], however, the mechanism is still unclear. The inset of Fig 4B showed the fluorescent image of **PEI-Suc** FONs taken under UV light of 365 nm, exhibiting bright blue fluorescence. Using 9,10-diphenylanthracene as the reference, the fluorescence quantum yield was estimated to be about 14% at an excitation wavelength of 365 nm. This intense fluorescence suggested that the as-prepared FONs were suitable for the potential cell imaging applications. It should be noted that PEI or sucrose alone did not show fluorescent emission, respectively, however, **PEI-Suc** FONs showed fluorescent emission, which might be attributed to the hydrolysis of sucrose and formation of Schiff base between PEI and glucose, as the C=N group was evidenced in the FONs by FT-IR and XPS experiment. And the formation of C=N bond would enhance the fluorescence efficiency significantly, which has been reported in the previous literature [21].

3.2 Stability of **PEI-Suc** FONs

The **PEI-Suc** FONs in aqueous solution exhibited a long-term homogeneous phase without any apparent precipitation at room temperature. In addition, the fluorescence intensity has no significant change during long-term storage for 8 weeks (Fig. 5A). Furthermore, no obvious attenuation of the fluorescence intensity was observed under continuous UV irradiation of 365 nm for 90 min (Fig. 5B). The above results demonstrated these **PEI-Suc** FONs had excellent nanoparticle stability and high photostability, which is beneficial for cell imaging application.

3.3 Biocompatibility of **PEI-Suc** FONs

The biocompatibility was conducted to evaluate the potential biomedical applications of **PEI-Suc** FONs. The effect of **PEI-Suc** FONs to A549 cells (adenocarcinomic human alveolar basal epithelial cells) was investigated after the cells were incubated with different concentrations of the FONs, and cell viability of **PEI-Suc** FONs to A549 cells was determined by cell counting kit-8 (CCK-8) assay. As

shown in Fig. 6, when the cells were incubated with 10-120 $\mu\text{g mL}^{-1}$ of **PEI-Suc** FONs for 8 h and 24 h, respectively, no obvious decrease of cell viability was found. Even when the concentration was raised up to 120 $\mu\text{g mL}^{-1}$, the cell viability value was still greater than 90%, indicating the low toxicity and excellent biocompatibility of the FONs, which can be used as a safe probe for in vivo applications.

3.4 Cell imaging applications of **PEI-Suc** FONs

Based on the merits of the **PEI-Suc** FONs, including high water dispersibility, intense fluorescence, excitation-dependent emission feature, excellent nanoparticle stability, high photostability, and excellent biocompatibility, the FONs were applied for multicolor living cell imaging. Cell imaging of the **PEI-Suc** FONs was explored, and the *in vitro* cellular uptake of **PEI-Suc** FONs was evaluated by Confocal Laser Scanning Microscope (CLSM) observation. As shown in Fig. 7B, when excited under laser of 405 nm, strong blue fluorescence could be observed in the cytoplasm region after only incubated with 10 $\mu\text{g mL}^{-1}$ of **PEI-Suc** FONs. When change the excitation wavelength of the laser to 458 nm, bright green fluorescence could be seen in the same region (Fig. 7C). It is found that **PEI-Suc** FONs could be uptaken by cells and mainly located in the cytoplasm and could not enter into the cell nuclei. The reason might be due to no specific cell tagging groups in the FONs, and the FONs were mainly internalized by cells through the endocytosis pathway [22]. This result indicated that the **PEI-Suc** FONs internalized in the cell could emit multicolor fluorescence under a laser of different wavelengths, and the excitation-dependent emission characteristic of the FONs enables them multicolor cell imaging, which has also been reported in the previous literatures [21, 23]. Owing to the intense fluorescence derived from the **PEI-Suc** FONs, strong fluorescent signal could be detected, which was highly beneficial for cell imaging.

4. Conclusions

In summary, a strategy of preparing FONs was developed for the first time via combining PEI and sucrose at mild condition. The reaction was conducted at 100 °C for 2 h to afford **PEI-Suc** FONs. The prepared FONs are well dispersed in water, and exhibited a spherical morphology according to the TEM result, and with an average size of 17 nm obtained from the DLS result. The FT-IR spectra and XPS results proposed the mechanism of the formation of **PEI-Suc** FONs might involve hydrolysis of sucrose and formation of Schiff base between PEI and glucose. The **PEI-Suc** FONs showed intense fluorescence with a quantum yield of about 14%, and the fluorescent emission of the FONs represented obvious excitation-dependent feature. We also demonstrated these **PEI-Suc** FONs had excellent

nanoparticle stability and high photostability. Biocompatibility evaluation and cell imaging application suggested that these FONs were successfully applied towards multicolor living cell imaging. More importantly, the preparation strategy in this work can extend to other water soluble polymers with amino groups and various carbohydrates, which would provide a new way to prepare more biocompatible fluorescent materials for biomedical applications.

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Figure Captions

Scheme 1 Preparation of **PEI-Suc** FONs from PEI and sucrose for cell imaging applications.

Fig. 1 (A) TEM image of **PEI-Suc** FONs dispersed in water, scale bar = 100 nm. (B) Size distributions of **PEI-Suc** FONs.

Fig. 2 (A) FT-IR spectra of PEI, sucrose, and **PEI-Suc**.

Fig. 3 XPS spectra of **PEI-Suc** FONs: (A) Wide range XPS spectrum showing the presence of carbon, nitrogen, and oxygen; (B) C 1s spectrum with peak at ~ 285 eV for sp² carbon atoms and shoulder peak at ~ 289 eV for sp³ carbon atoms; (C) N 1s spectrum with peak at ~ 400 eV. (D) O 1s spectrum with peak at ~ 532 eV.

Fig. 4 (A) UV absorption spectrum of **PEI-Suc** FONs dispersed in water, inset is the visible image of **PEI-Suc** FONs in water; (B) PL emission spectra (right) of **PEI-Suc** FONs dispersed in water under different excitation wavelengths and the excitation spectrum (left), inset is the fluorescent images of **PEI-Suc** FONs taken under 365 nm UV light.

Fig. 5 (A) The change of fluorescence intensity of **PEI-Suc** FONs at different storage time. (B) The change of fluorescence intensity of **PEI-Suc** FONs at different illumination time under 365 nm UV light.

Fig. 6 Cell viability of A549 cells incubated with different concentrations of **PEI-Suc** FONs for 8 h and 24 h.

Fig. 7 CLSM images of A549 cells incubated with 10 µg mL⁻¹ of **PEI-Suc** FONs for 3 h. (A) bright field, (B) excited with 405 nm laser, (C) excited with 458 nm laser, (D) merged image of A and B. Scale bar = 20 µm.

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