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Fabrication of water-dispersible and biocompatible red fluorescent organic nanoparticles via PEGylation of aggregate induced emission enhancement dye and their cell imaging applications

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ABSTRACT

PEGylated red fluorescent organic nanoparticles (FONs) with aggregate induced emission enhancement (AIEE) properties have been prepared via self assembly of a cyano-substituted diarylethene derivate dye (C18-R) and synthetic copolymers, which were obtained by reversible addition-fragmentation chain transfer (RAFT) polymerization using stearyl methacrylate and poly(ethylene glycol) methacrylate as monomers. Thus obtained FONs were characterized by a series of techniques including transmission electron microscopy, Fourier transform infrared spectroscopy and fluorescent spectroscopy. To explore their potential biomedical applications, biocompatibility and cell uptake behavior of these red FONs were subsequently investigated. We demonstrated that FONs showed uniform morphology, suitable particle size (70–90 nm), high water dispersibility, strong red fluorescence and excellent biocompatibility, making them promising for bioimaging applications.

1. Introduction

The development of novel fluorescent nanoparticles has been the subject of intensive research in recent years because of the great interest in their applications in a variety of fields ranging from biological labeling to environmental analysis and optical devices [1–6]. Various fluorescent nanoparticles based on inorganic and organic components have thus been prepared and extensively investigated especially for biomedical applications [7–11]. Among them, inorganic nanomaterials such as semiconductor quantum dots (QDs), carbon dots, fluorescent silica nanoparticles and metal nanocrystal have received considerable attention for bioimaging applications and have been regarded as alternative for conventional organic dyes and fluorescent proteins owing to their excellent photostability, tunable photoluminescence, easy of synthesis and low cost [12–15]. However, potential toxicity is still a big challenge for the biomedical applications of most fluorescent inorganic nanoparticles (FINs). It has been demonstrated that QDs were toxic to living organisms due to their heavy metal compositions. On the other hand, most of FINs is nonbiodegradability, which will inevitably arouse security issues for their long term in vivo applications. Therefore, the searching of novel fluorescent nanoprobes which could overcome these obvious shortcomings of FINs is of great scientific significance.

Compared with FINs, fluorescent organic nanoparticles (FONs) have recently attracted increasing research interest for biomedical applications because of their flexible synthetic approaches of small organic molecules, biodegradable potential, biocompatible and non-toxic properties [16–20]. Many types of FONs based on conjugated polymers, polydopamine nanoparticles, aggregate induced emission (AIE) and aggregate induced emission enhancement (AIEE) dyes have been reported [21–33]. Among them, AIE/AIEE dyes have been emerging as promising candidates for fabrication of FONs due to their extraordinary anti-aggregation caused quenching (anti-ACQ) effect, strong fluorescent without photobleaching and photoblinking. Since Tang et al. reported AIE phenomenon in

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2001 [23], a variety of AIE/AIEE dyes including tetraphenylethene [34–39], siloles [40,41], cyano-substituted diarylethene [42], triphenylethene [43–46], distyrylanthracene [47–50] derivatives have been synthesized and utilized for fabrication of AIE/AIEE based FONs for bioimaging applications. However, the fluorescence excitation and emission of most AIE dyes are felled into visible region, which is not suitable for practical bioimaging applications due to the strong autofluorescent of living organisms in this region. It is therefore development of AIE/AIEE dye based FONs, which emitted in the red to near-infrared range are highly desirable for their practical biomedical applications.

In this work, we report a facile strategy for preparation of red FONs via self assembly of a cyano-substituted diarylethene derivate dye (named as **C18-R**) and synthetic copolymers, which were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization using stearyl methacrylate (SMA) and poly(ethylene glycol) methacrylate (PEGMA) as monomers. To obtain the FONs, **C18-R** was first dissolved in THF, and then was dropwisely added into the copolymer-contained aqueous solution under ultrasonication. During the removal of THF, **C18-R** was encapsulated by the synthetic polymers through hydrophobic interaction between **C18-R** and hydrophobic segment (SMA) of synthetic copolymers (Scheme 1), thus obtaining PEGylated **C18-R-PEG** FONs. To exploit their potential biomedical applications, biocompatibility and cell uptake behavior of **C18-R-PEG** FONs were further investigated.

2. Experimental procedure

2.1. Materials and characterization

Thiophene, 3-(bromomethyl)heptane, thiophene-2-carboxylic acid, thionyl chloride, dimethylamine, *n*-butyllithium, stannic chloride, 2-(4-methoxyphenyl)acetonitrile, 2-phenylacetonitrile, 2-(4-(trifluoromethyl)phenyl)acetonitrile, tetrabutylammonium hydroxide (0.8 M in methanol) purchased from Alfa Aesar were used as received. Tetrahydrofuran (THF) was distilled from sodium/benzophenone. Ultra-pure water was used in the experiments. Intermediates 1–3 were prepared according to previous reports and further used for synthesized **C18-R** (Scheme 2) [51,52]. The hydrophobic monomers (SMA) were purchased from Aladdin (Shanghai, china, 96%) and hydrophobic monomers (PEGMA, MW: 950 Da, J&K chemical, 98%) were used as received. The chain transfer agent (CTA) was synthesized according to our previous report [53]. All other agents and solvents were purchased from commercial sources and used directly without further purification. Tetrahydrofuran (THF) was distilled from sodium/benzophenone. Ultra-pure water was used in the experiments.

2.2. Synthesis of 4

3 (458 mg, 0.79 mmol) was dissolved in anhydrous THF (40 mL) under Ar gas, then the temperature of the solution was cooled to −78 °C, afterwards, 0.79 mL *n*-BuLi (2.5 mol/L in THF) was added, allowing the mixture to react for 1 h with the temperature raising to room temperature, and then cooled to −78 °C again, 0.3 mL DMF was added dropwise to the solution for 0.5 h, the solution was stirred at room temperature for 3 h, then water was added to quench the reaction. The resulting mixture was extracted by dichloromethane, purification was carried out by column chromatography on silica gel using petroleum ether–dichloromethane (2:1, V/V) as the eluent to obtain pure compound 4 as orange-red solid (0.35 g, yield 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.89–1.00 (m, 12H, —CH_3), 1.29–1.49 (m, 16H, —CH_2—), 1.62–1.78 (m, 2H, (methylene)3C—H), 2.89 (d, 4H, J = 6.8 Hz, thienyl—CH_2—), 6.95 (d, 2H, J = 3.6 Hz, thienyl—H), 7.34 (d, 2H, J = 3.6 Hz, thienyl—H), 8.36 (s, 2H, thienyl—H), 10.10 (s, 2H, —CHO); MS (FAB) calcd. for $C_{36}H_{42}O_2S_4$ 634, found 634.

2.3. Synthesis of C18-R

A solution of 4 (0.12 g, 0.19 mmol) and 5 (0.16 g, 0.42 mmol) in ethanol (10 mL) was stirred at room temperature. Then terabutyl ammonium hydroxide solution (0.8 M, 5 drops) was added and the mixture was heated to reflux for 2 h precipitating a dark red solid. The reaction mixture was cooled to room temperature and filtered, washed with ethanol for several times obtaining a dark red solid **C18-R** (0.22 g, yield 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.85–0.97 (m, 18H, —CH_3), 1.24–1.49 (m, 76H, —CH_2—), 1.66–1.84 (m, 2H, (methylene)3C—H), 2.87 (d, 4H, J = 6.8 Hz, thienyl—CH_2—), 3.96 (t, 4H, J = 6.4 Hz, O—CH_2—), 6.86–6.96 (m, 6H, Ar—H), 7.35 (d, 2H, J = 3.2 Hz, Ar—H), 7.47–7.64 (m, 6H, Ar—H), 7.99 (s, 2H, Ar—H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.3, 146.6, 140.0, 139.7, 136.9, 135.9, 132.2, 132.1, 129.9, 129.6, 128.6, 128.3, 127.6, 127.4, 127.3, 127.0, 126.2, 126.1, 125.6, 124.7, 117.7, 115.3, 114.8, 110.7, 70.7, 68.3, 41.6, 41.4, 34.8, 34.4, 32.6, 32.0, 30.6, 29.8, 29.5, 29.3, 29.0, 26.1, 25.8, 23.1, 22.8, 14.4, 14.3, 14.2, 11.0, 10.9; HRMS calcd. for $C_{88}H_{124}N_2O_2S_4$, [M+Na]+: 391.8438, found 1391.8250.

2.4. Preparation of copolymers

The copolymers were synthesized as described in our previous report [53]. In a typical experiment, PEGMA (2.66 g, 2.8 mmol), SMA (0.41 g, 1.2 mmol), the chain transfer agent (CTA) (21.0 mg, 0.08 mmol), AIBN (4.0 mg, 0.016 mmol) and toluene (4.0 mL) were introduced in a schlenk tube with a magnetic stir bar, and purged by nitrogen flow for 30 min. The final reaction mixture was put into an oil bath maintained at 70 °C for 20 h (Scheme 3). At the end of the polymerization, the purified polymer was obtained via precipitation from methanol to diethyl ether for three times, and then dried under vacuum for further characterization. All polymers in current report are obtained with the same approach. Based on the feed molar ratios of PEGMA to SMA, these polymers were named as PEG-3 (PEGMA/SMA = 3:7) and PEG-7 (PEGMA/SMA = 7:3). The absorption of CTA and copolymers at 302 nm was measured by UV–vis spectroscopy to determine polymers Mns. Based on the UV–vis spectra, the Mns of PEG-3 and PEG-7 are 43 000 Da and 60 100 Da, respectively.

2.5. Preparation of C18-R-PEG FONs

The preparation of **C18-R-PEG** FONs was carried out as follows. Approximately 20 mg of **C18-R** dyes was dissolved in 10 mL of THF was slowly added into 20 mL of H_2O contained 80 mg of copolymers in a 100 mL vial under ultrasonic. And then the mixture was evaporated to completely remove the organic agent (THF) on a rotary evaporator at 40 °C. And then red color suspensions were obtained, implying the successful formation of **C18-R-PEG** FONs. To remove the excess copolymers, **C18-R-PEG** aqueous dispersion was treated by repeated centrifugal washing process for thrice. Finally, the sediment was collected and utilized for further characterization and biological experiments. The concentrations of **C18-R-PEG** nanoparticles were the added **C18-R** dyes.

2.6. Cytotoxicity of C18-R-PEG FONs

Cell morphology observation is a simple and important experimental procedure for biocompatibility evaluation. In this work, the effects of **C18-R-PEG** FONs to human lung adenocarcinoma (A549) cells were examined according to our previous reports [54–57]. Briefly, cells were seeded in 6-well microplates at a density of