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A high brightness probe of polymer nanoparticles for biological imaging

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ABSTRACT

Conjugated polymer nanoparticles (CPNs) with high brightness in long wavelength region were prepared by the nano-precipitation method. Based on fluorescence resonance energy transfer (FRET) mechanism, the high brightness property of the CPNs was realized by four different emission polymers. Dynamic light scattering (DLS) and scanning electron microscopy (SEM) displayed that the CPNs possessed a spherical structure and an average diameter of ~75 nm. Analysis assays showed that the CPNs had excellent biocompatibility, good photostability and low cytotoxicity. The CPNs were bio-modified with a cell penetrating peptide (Tat, a targeted element) through covalent link. Based on the entire wave fluorescence emission, the functionalized CPNs1–4 can meet multichannel and high throughput assays in cell and organ imaging. The contribution of the work lies in not only providing a new way to obtain a high brightness imaging probe in long wavelength region, but also using targeted cell and organ imaging.

1. Introduction

Recently, fluorescence nanoscale materials have been widely attended and fast developed in biological applications such as biosensors, fluorescence imaging, delivery of gene and drugs, diagnoses and treatments for diseases [1–6]. These nanomaterials included inorganic semiconductor quantum dots (SQDs), conjugated polymer dots (CPDs), conjugated polyelectrolyte dots (CEDs), and carbon dots (CDs), etc. [7–10]. Among there nanomaterials, conjugated polymer nanoparticles (CPNs) were regarded as the most promising materials in the multi-disciplinary fields for fluorescence imaging, clinical diagnosis and treatment of disease. Conjugated polymer nanoparticles not only possessed excellent optic/electro properties and amplified function of conjugated polymers (CPs), but also combined the size effect of nanoparticles [11–20]. Especially, the CPNs can overcome the defects (such as hydrophobicity and hard purification) of CPs in biological applications. To date, conjugated polymer nanoparticles have been applied in various biological aspects, and they displayed some unique properties such as high brightness, good photostability, low cytotoxicity, excellent biocompatibility and multicolor emissions, etc. [21,22]. However, the development limitation of CPNs in biological field (especially in bioimaging) lied in the low fluorescence in long wavelength regions of the reported CPNs. It is well known that the long wavelength emission of CPNs was very important for bioimaging application [23–30]. Firstly, the kind of CPNs had a high penetration capacity to target cells and organs. Secondly, they could reduce the auto-fluorescence from biomolecules in biological systems. Lastly, minimum photo-damage for biological samples was also one of this kind CPNs advantages. Undoubtedly, the kind of CPNs will develop a new plat for chemists and biologists to explore complex biological processes at the molecular level. Many efforts had been made to obtain CPs or CPNs with a long wavelength emission in the past five years. However, due to their big planar structures and easy aggregation behaviour, the kind of emitters (CPs or CPNs) exhibited a low fluorescence emission [31,32]. Therefore, it is an urgent challenge for researchers to obtain long wavelength emitters with high brightness.

To get the CPs with a long wavelength emission, the first way is to elaborately design and synthesize fluorophores monomers, further polymerization. However, the process was very difficult because of the long multi-step reaction and complex chemical modifications. Compared with the first way, fluorescence resonance energy transfer (FRET) strategy is simple and feasible [33]. For a typical FRET process, the energy from a donor to an acceptor is the intermolecular long-range dipole-dipole coupling. The FRET process depended on a distance (Förster radius) of the donor and the acceptor [34]. Conjugated polymers (CPs) based on FRET not only took place the red-shift of excitation and emission wavelengths, but also improved the fluorescence efficiency [35–38]. In view of the feature of CPs by FERT, many biomaterials and ensembles had been developed for fluorescence imaging, biosensors, gene detection and photodynamic therapy, etc. [4,39–43].

Currently, the CPNs used in fluorescence imaging mainly located in the cytoplasm through nonspecific cellular uptake way. The action method was no selectivity and targeting [44–47]. For the transfer efficiency and speed, the method also was low and slow. So, it was desired of developing the kind of CPNs with biomolecules modified, especially

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through covalent bonding mode. In the aspect, Wu and co-workers made a very big contribution. They successfully connected the streptavidin or antibody to CP dots by a covalent mode and realized targeted tumor cells and organs imaging [9,48]. Similarly, our groups' research developed multicolor CPNs bioconjugated with antibodies, which could target cells imaging and recognize different tumor cells [49]. Regrettably, the emitter in long wavelength region exhibited a very low fluorescence emission. To improve the efficiency of target and permeation, we recently exploited yellow emission CPNs modified with antibody and peptide. The functionalized CPNs not only can target different parts of cells, but also may damage the tumor cells by a photodynamic mode [50].

In this work, we prepared multiple wavelength emission CPNs1–4 with four fluorene derivatives as a core and carboxyl groups in the surface by a nano-precipitation method. Based on REET mechanism, the CPNs displayed higher fluorescence emission in long wavelength region. To perform the biological applications, the CPNs1–4 were modified with cell penetrating peptide (Tat) by a covalent binding way. The fluorescence imaging of the functionalized CPNs for tumor cells and organs were investigated by confocal laser scanning microscopy.

2. Materials and Methods

2.1. Materials and Instruments

Unless otherwise stated, all chemical reagents were obtained from commercial suppliers and used without further purification. Four fluorene derivants (PFP, PFO, PFBT and PFBO) were provided by Key Laboratory of Organic Solids Institute of Chemistry of Chinese Academy of Sciences. Polymer PSPEGCOOH was purchased from Polymer Source of Canada. The peptide (Tat, Lys-Lys-Lys-Arg-Lys-Val-Ala-Ala-Agr-OH) was obtained by GL Biochem. Ltd. of China (Shanghai). Other chemicals were purchased from Aldrich (Steinheim, Germany). The UV–Vis absorption and fluorescence emission spectra of these CPNs were determined on HITACHI UH5300 and F-4600 spectrophotometers, respectively. Accordingly, the excitation and emission slit widths were both 10.0 nm. The water used in nanoparticle preparation and imaging assays was purified by a Millipore filtration system. A microplate reader (BIO-TEK Synergy HT, USA) was used to detect the absorbance for MTT assays according to various excitation wavelengths. Dynamic light scattering (DLS) tests of the CPNs were taken on a Nano ZS (ZEN3600) system. Scanning electron microscope (SEM) images were measured on a Hitachi S-4800 scanning electron microscope. The fluorescence images for tumor cells and mouse organs were recorded with a confocal laser scanning biological microscope (FV1000-IX81, Olympus, Japan).

2.2. Preparation and Functionalization of CPNs

All used water was purified by a Millipore filtration system. The CPNs with carboxyl groups on the surface were prepared by a nano-precipitation method. A mixture of 50 μg/mL conjugated polymer (for example PFP), 50 μg/mL PSPEGCOOH and 10 mL THF was stirred uniformly. Then, the mixture was quickly added to 20 mL Mill-Q water under ultrasonication for 5 min. The mass of organic solvent (THF) in the dispersion was removed by inletting nitrogen continuously at room temperature. Lastly, the CPNs dispersion was concentrated to 10 mL by heating under nitrogen. For the CPNs1–4/PSPEGCOOH, the original mixture was comprised of 30 μg/mL (PFP), 100 μg/mL (PFO), 50 μg/mL (PFBT), 50 μg/mL (PFBO) and 50 μg/mL (PSPEGCOOH) in THF. The modifications of CPNs4/PSPEGCOOH and CPNs1–4/PSPEGCOOH by the peptide (Tat) were performed by condensation reaction of amino and carboxyl groups under NHS-SO_3Na activation and EDCI catalysis.

2.3. Characterization

The characterizations of CPNs were determined by SEM and DLS techniques. For SEM measurements, 10 μL of the CPNs dispersion was placed on a clean silicon slices. Then, the dispersion was quickly frozen and evaporated the water through freeze drying. The morphology of CPNs1–4/PSPEGCOOH was detected by a Hitachi S-4800 scanning electron microscope. In the DLS tests, after a certain amount of the CPNs dispersion was added the detector, the sizes of CPNs were reported by Nano ZS (ZEN3600) system.

2.4. Fluorescence and UV–Vis Measurements

All used water was Mill-Q water in advance purification. The UV–Vis absorption and fluorescence emission spectra were determined with the preparation CPNs dispersion. For the fluorescence spectra, 2.0 mL CPNs dispersion was placed in a quartz cuvette with 1 cm path. The excitation and emission slits in the experiment both were 10 nm. All measurements were proceeded at room temperature.

2.5. Theoretical Calculation

The geometries and electron density distributions of the HOMO and LUMO energy levels of these conjugated polymers were calculated through density functional theory (DFT) and Gaussian 09 software. The geometries of PFP, PFO and PFBT polymers were optimized under the DFT/B3LYP/6-31G(d) mode. The geometry of PFBO was obtained under the DFT/B3LYP/3-21G mode.

2.6. Cell Culture

The human lung adenocarcinoma cell line (A549) was obtained from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). The culture medium was Roswell Park Memorial Institute (RPMI) with 10% Fetal bovine serum (FBS) at 37 °C, 5% CO_2 humidified atmosphere. The tumor cells were placed in a Petri dish (35 mm × 35 mm) with a glass bottom. The wash buffer for the tumor cell was PBS buffer in the experiment.

2.7. Assay of Cell Viability

In RPMI medium, the 96-well tissue culture plates were used to seeded A549 cells and until complete adherence. After adding various quantities of CPNs/PSPEGCOOH, these cells were sequentially incubated with RPMI for 24 h. After Washing the cells three times with PBS, 1.0 mg/mL MTT (100 μL/well) was introduced these culture plates. Then, the cells were cultured at 37 °C for 4 h continuously. After removed the media, 150 μL of DMSO was added to per well, and shook for 5 min. The absorbance values according different wavelengths were recorded on the microplate reader (BIO-TEK Synergy HT, USA).

2.8. Cell Imaging In Vitro

Firstly, A549 cells were cultured at 5% CO_2 humidified atmosphere and 37 °C to until the density reached about 80% on a confocal dish (Coverglass Bottom Dish). Then, the media with 2.5 μg/mL CPNs1–4/PSPEGCOOH, CPNs4/PSPEGCOTat or CPNs1–4/PSPEGCOTat were added to the dish and sequentially cultured for 3 h at 37 °C, respectively. After washing the cells three times with PBS buffer, the cells were fixed with 4% paraformaldehyde at room temperature for 20 min. Lastly, the cells were washed two times and covered with PBS buffer. The cell imaging in vitro was reported by fluorescence confocal microscope. The fluorescence collection range was 410–500 nm for blue emission under 405 nm excitation. For red fluorescence emission, the collection range was 550–650 nm under 543 nm excitation.