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A single-step emulsion approach to prepare fluorescent nanoscale coordination polymers for bioimaging†

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A crystalline nanoscale coordination polymer (NCP) based on fluorene and Zr^{4+} was synthesized through a simple microemulsion method. The emulsion method is facile and convenient for NCP production, the nanoparticles are fluorescent and thermal-stable, and most importantly, they are in nanoscale thus making them applicable for bioimaging.

Luminescent nanomaterials have drawn great interest in bioscience such as in bioimaging, as drug carriers, and for disease detection.[1] In luminescence bioimaging, luminescent nanomaterials are used to label a molecule of interest and to render luminescent signals. Many new luminescent nanomaterials, have been synthesized for bioimaging application including semiconductor nanocrystals,[2] upconversion nanophosphors,[3] quantum dots[4] and other nanoparticles.[5–8] Among them, coordination polymers (CPs), also called metal–organic frameworks, are attractive not only for their porous structures and high surface areas, but also for the convenience of them to be designed and incorporated with function groups which made CPs have potential applications in catalysis,[9–11] gas storage,[12,13] separation[14] and bioscience.[15,16] However, CPs particles are usually in micrometer scale, which limit their application. Recently, Lin's group reported the ability to scale down the size of CPs to nanoscale,[17] thus made the luminescent CPs an excellent material for biological applications.[18–21]

Conventionally, CPs are synthesized *via* time-consuming hydrothermal or solvothermal methods, these methods require several hours or days for crystallization and nanoparticles formation. Other approaches involve alternative energy sources such as microwave irradiation or ultrasound usually decreased the crystallization time, but required special power-consuming apparatus. It is highly desirable to develop new method to prepare the CPs in simple and mild condition. Very recently, several groups have reported some new methods to make CPs, like spray-dry strategy[22] and microfluidic approach.[23]

Microemulsion method (MEM) is usually used for the preparation of polymeric nanoparticles. It is easy and convenient, and the nanoparticles prepared by MEM usually have narrow size distribution.[24] Very recently, Eddaoudi *et al.* reported a single-step emulsion-based technique to assembly of CPs into 3D hollow superstructures.[25]

Polyfluorenes are of interest and currently being investigated to use in light-emitting diodes, field-effect transistors and plastic solar cells.[26–28] Fluorene-based materials have been studied in bioscience because of their stability and safety to living cells.[29–31] Some reports on the conjugated polymers based on fluorene for biomarkers and detection of proteins and DNA were published in last several years.[32–35]

Herein, we synthesized novel nanoscale coordination polymers (NCPs) with microemulsion method using Zr^{4+} and fluorene derivatives. The NCPs were characterized by thermogravimetric analysis (TGA), powder X-ray diffraction (PXRD), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Then the bioimaging experiment was carried out for they are fluorescent and in nanoscale which is easy to endolysis by living cells.

Scheme 1 shows the preparation of the ligand and NCPs. The ligand (2,7-(4-carboxyl-benzene)-9,9′-dioctyl-9*H*-fluorene) was prepared through Suzuki coupling reaction and subsequent hydrolysis reaction. The structures were confirmed by ^{1}H-NMR (Fig. S1, ESI†). Then the ligand and $ZrCl_4$ were dissolved in DMF with a small amount of trifluoroacetic acid (TFA) and hydrochloric acid (HCl) to modulate the crystallinity of the nanoparticles, then the mixture was dropped into a heated silicon oil bath to get the NCPs. Silicon oil is immiscible with DMF and used to form emulsion drops, in which the particles were prepared after aggregation, nucleus and crystallization[36] (see the ESI† for detail).

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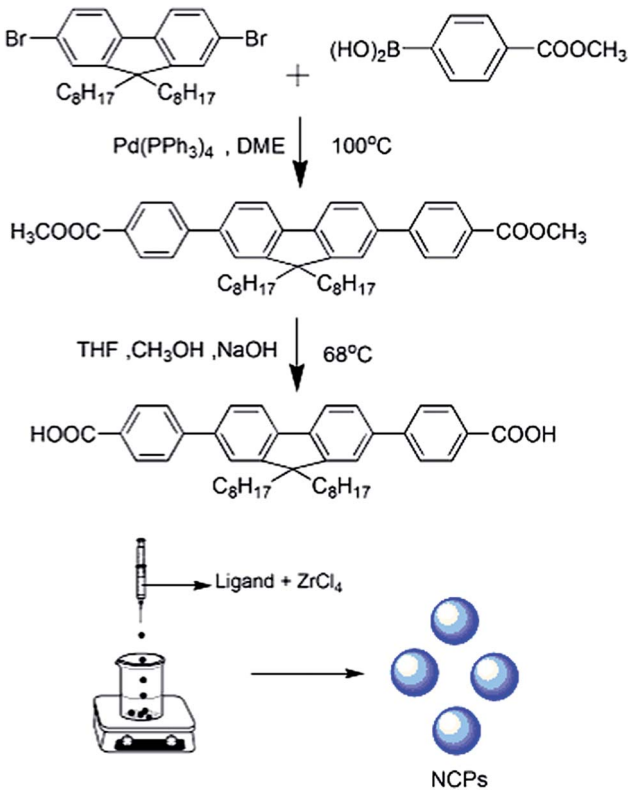

Scheme 1 Preparation of ligand and NCPs.

The SEM (a and b) and TEM (c) of the NCPs were presented in Fig. 1. The images show the particles were in good dispersion, and in nanoscale with diameter of about 50 nm, which is easy to endocytosis by living cells for bioimaging application. We can see that the particles are in nearly the same sphere morphous from the SEM pictures.

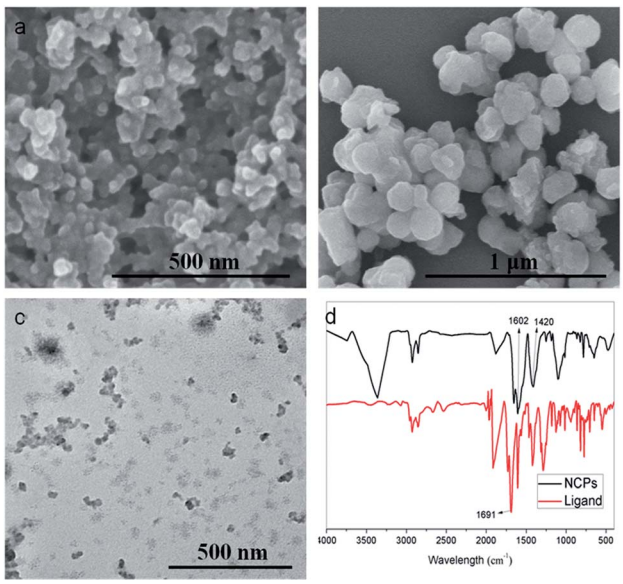

Fig. 1 SEM (a and b) and TEM (c) of the NCPs. FT-IR Spectroscopy (d) of the ligand (red) and the particles (black).

Fig. 1d shows the FT-IR of the ligand and the nanoparticles. The peaks at 2900 cm^{-1} are due to C–H stretching vibration of the C_8H_{17} chain of the fluorene for both the ligand and the particles. Compared with the ligand, the missing of the peak at 1691 cm^{-1} of the NCPs is responsible for the coordination bond formation in coordination polymers. Instead, the appearance of two new peaks at 1602 cm^{-1} and 1420 cm^{-1} are responsible for antisymmetric and symmetric carboxylate stretching vibrations, the value between 1602 cm^{-1} and 1420 cm^{-1} is less than 200 cm^{-1}, this indicates the ligand is connected to Zr^{4+} through bidentate coordination state.[37–40]

Since the NCPs are crystalline, as indicated by the PXRD shown in Fig. 2a, we decided to study the structure of our NCPs. From Fig. 2a, we can see the PXRD pattern of our NCPs fit well but a little blue shifted compared to that of UiO-66, so we concluded our NCPs would share the same structure with UiO-66,[41] which is consistent with the analysis of FT-IR. And we simulated the structure of our NCPs as seen in Fig. 2c. The possible reason of the slight difference is that the unit cell volume of our NCPs is larger than UiO-66, the diffraction angles shift to the small angles. We assumed there are two reasons for this: (1) the ligand is different; our ligand is much longer compared to terephthalic acid, which is the ligand of UiO-66, and the octane chains of our ligand could increase the cell volume. (2) We added TFA and HCl as modulation to control the crystallization, and the TFA could coordinate to Zr^{4+},[42] then left after activated, thus would affect the PXRD pattern.

We also studied the TGA of the nanoparticles, the result is shown in Fig. 2b. The weight loss of the material is steady decreased from room temperature to about 300 °C due to the solvent loss. The weight loss from 300 to 500 °C is because of the ligand decomposing. And from 500 to 630 °C, the rate of weight loss decreased and the finally get to a steady state. This is because that the final substance is the very stable zirconium oxide. The TGA result shows our NCPs are as stable as

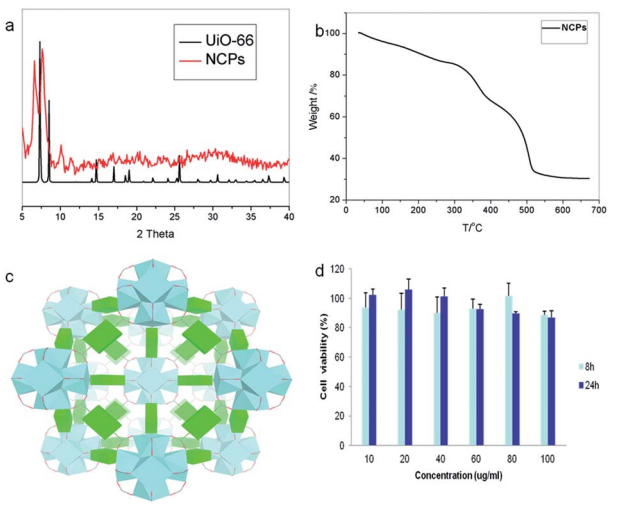

Fig. 2 (a) PXRD of as synthesized NCPs (red) and UiO-66 (black). (b) TGA. (c) Simulated structure of NCPs, the green parts represent the ligand, colors of atoms O (red), Zr (light blue); MTT (d) of HeLa cells after NCPs endocytosis.

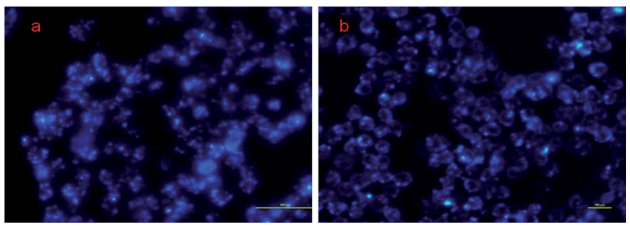

Fig. 3 Microscopic images of the cells. The scale bars are 500 μm and 100 μm for (a) and (b), respectively.

UiO-66.[43,44] Moreover, the amount of the Zr^{4+} calculated from the result of TGA is 22.56 wt%, which fits well with the result of ICP-MS, 23.08%.

The particles remain fluorescence when dispersed in water (as shown in Fig. S2 and S3, ESI†) and common organic solvents. They are possible used for bioimaging for the fluorescence and nanoscale size. Determination of the biocompatiblitity of NCPs is very important before using them in living cell.[45,46] As shown in Fig. 2d, no obvious cytotoxicity to human breast cancer cells (HeLa) is observed *via* cell viability examination. Even with a concentration of NCPs up to 100 μg mL^{-1}, the cell viability is still greater than 90% after 24 h, demonstrating their excellent biocompatibility. Then the bioimaging experiment was further exploited to demonstrate their biomedical applications using HeLa cells. Fig. 3a and b confirmed our nanoparticles are in the cytoplasm of the cells, which indicated by Lin's and Petoud's groups.[17,47] The signal of the nanoparticles is high under microscopic with irritation. To further confirm the NCPs remain intact within HeLa cells, we carried out the control experiment with the ligand as the bioimaging agent, the pictures are presented in Fig. S4 (ESI).† Because the ligand is not soluble in water environment so it aggregates into large particles, which is different with the NCPs. We can also see that the cells were in good shape and condition under different resolutions.

In summary, we have synthesized a nanoscale coordination polymer material through a convenient microemulsion method. The nanoparticles were characterized through FIRT and PXRD, which indicated the NCPs formed through coordination bond and were crystalline. SEM and TEM pictures confirmed the size of the particles were in good distribution and in nanoscale which made them possible for endocytosis by living cells. The fluorescence of the NCPs in HeLa cells after endocytosis for bioimaging confirmed our idea for the possible application on bioscience of the NCPs.

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