Preparation of Autofluorescent Polyacrylamide Nanoparticles

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Abstract: Nowadays, tumor imaging is widely used in biomedical field. However, the materials of imaging is rarely and strict. This study developed a method for preparing autofluorescent polyacrylamide nanoparticles (NPs). The polyacrylamide NPs were treated with glutaraldehyde, and then removed excess glutaraldehyde through ultrafiltration, glycine neutralization and ethanol precipitation, respectively. Based on this method, the polyacrylamide NPs showed strong near-infrared fluorescence (NIRF) at the emission wavelength of both 720 and 820 nm. Additionally, the nanoparticles were efficiently internalized into RAW264.7 cells and displayed strong green and red fluorescence observed with fluorescence microscope. Therefore, the NPs are expected to be applied to tumor imaging in future.

1. Introduction

In recent years, optical imaging is widely used in medical diagnosis and molecular biology, which will have an important impact on the prevention and treatment of cancer and other deadly diseases [1, 2]. However, it is necessary to find a fluorescein carrier with good biocompatibility [3]. The polyacrylamide NPs are an important water-soluble polymeric material, which can form a stable colloidal dispersion system in water, showing excellent biocompatibility [4]. Furthermore, in the cross-link process of using the carrier to construct the fluorescence probe, which usually affects the intrinsic properties of fluorescent dyes and carriers. Therefore, it is necessary to explore a material with spontaneous fluorescence properties. Many researchers have used glutaraldehyde to construct organic nanomaterials with spontaneous fluorescence properties [5-8], but their emission fluorescence can not meet the imaging needs for only visible range. The paper constructed a spontaneous fluorescence polyacrylamide NPs with NIFR properties, indicating potential wide application in the optical imaging.

2. Methods

2.1 Preparation of polyacrylamide NPs

Polyacrylamide NPs were prepared by reversed-phase microemulsion system. 90 mL n-hexane was firstly add to a dry 250 mL round bottom flask, and stirred in argon for 10 min. Then add 3.2 g surfactant AOT and 6.3 mL Brij30, stir mixture for 40 minutes under argon protection. Adding 80 μL new prepared ammonium persulfate (APS) aqueous solution (10% w/v) and 80 μL TEMED initiation polymerization. After that add the monomer solution, which includes 530 mg acrylamide, 30 mg APMA, 160 mg MBA and 2ml ultra-pure water, to the flask and the reaction was 3h under the protection of argon. After the polymerization reaction, transfer to the rotary evaporator to remove n-hexane. The final nanoparticle suspension was obtained through ethanol precipitation and filtration. The hydrodynamic size and zeta potential of polyacrylamide NPs suspension were analyzed using the Zetasizer Nano particle analyzer.

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2.2 Glutaraldehyde deals with polyacrylamide NPs

The addition of 400 μL glutaraldehyde (25 %) was added to the 1600 μL nanoparticles, ultrasonic reaction under 37 ℃ for 1 h, After ultrasound, pour into the dialysis bag, dialysis 12 h. The above treated samples were concentrated in the ultrafiltration tube, and then the 5500 rpm centrifuge was removed for 30 min (the supernatant is named S1). In addition, add 2000 μL glycine solution (1 M), 1500 μL H_2O. After 37 ℃ water bath overnight, with ethanol precipitation except the supernatant (S2), with 4000 μL water hung heavy precipitation final suspension (NPs-f).

2.3 Transfection cells

First suck out cell culture from the orifice plate, and then clean with PBS 2-3 times, add 500 μL/hole cell cultures, and add 100 μL nanoparticles to the corresponding hole, incubate at 37 ℃ for 4 hours. After the cell was fixed at room temperature for 20 min with 4 % paraformaldehyde, DAPI staining was used to dye the nucleus, and then the cells were imaged with fluorescence microscopy.

3. Results

3.1 Characterization of polyacrylamide NPs

The polyacrylamide NPs possessed a great dispersibility through the transmission electron microscopy (TEM) observation (Figure 1A). The average particle size in aqueous solution was about 79 nm with dynamic light scattering (DLS) in a particle size analyzer (Figure 1B). This nanoparticle can be used for subsequent experiments for their great dispersibility and size uniformity in water.

3.2 Glutaraldehyde deals with polyacrylamide NPs

The polyacrylamide NPs treated by glutaraldehyde presented deeper yellow under the white light (Figure 2A). The NPs showed slight yellow with the ultra-filtered and glycine added (Figure 2B). Novelly, the NPs displayed strong NIFR at the emission wavelength of both 720 and 820 nm (Figure 2C). Additionally, it was found that the NIRF at the emission wavelength of 720 nm was much stronger than that at 820 nm (Figure 2C).

3.3 Cellular internalization

The cellular internalization of the nanoparticle was detect by the common fluorescence microscopy, It was found that the nanoparticles could be efficiently internalized into RAW264.7 cells and displayed strong green and red fluorescence with fluorescence microscope, respectively (Figure 3). The results proved that the cells labeled by polyacrylamide NPs showed good imaging effect with both visible and infrared fluorescence.

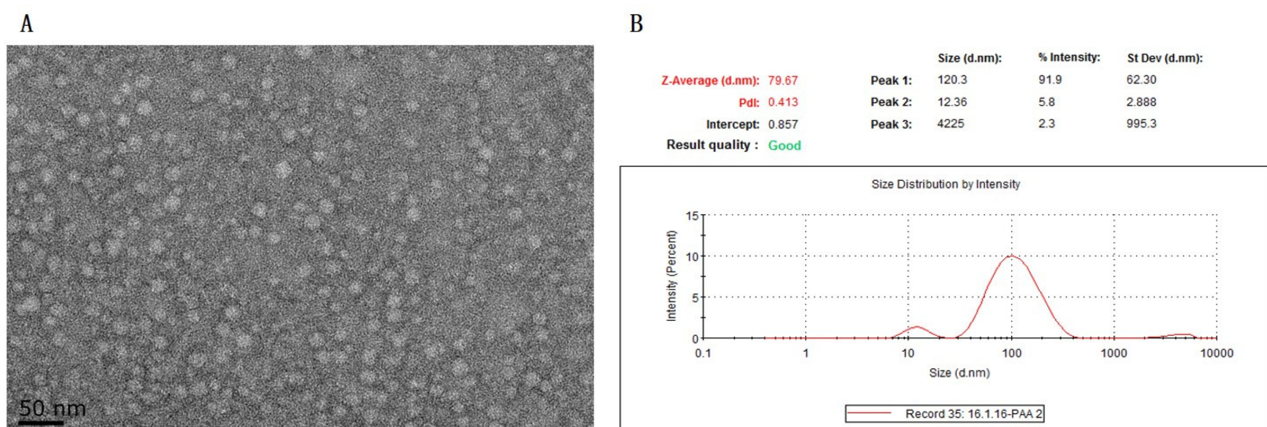

Figure 1

Fig.1 Characterization of polyacrylamide NPs with SEM and DLS

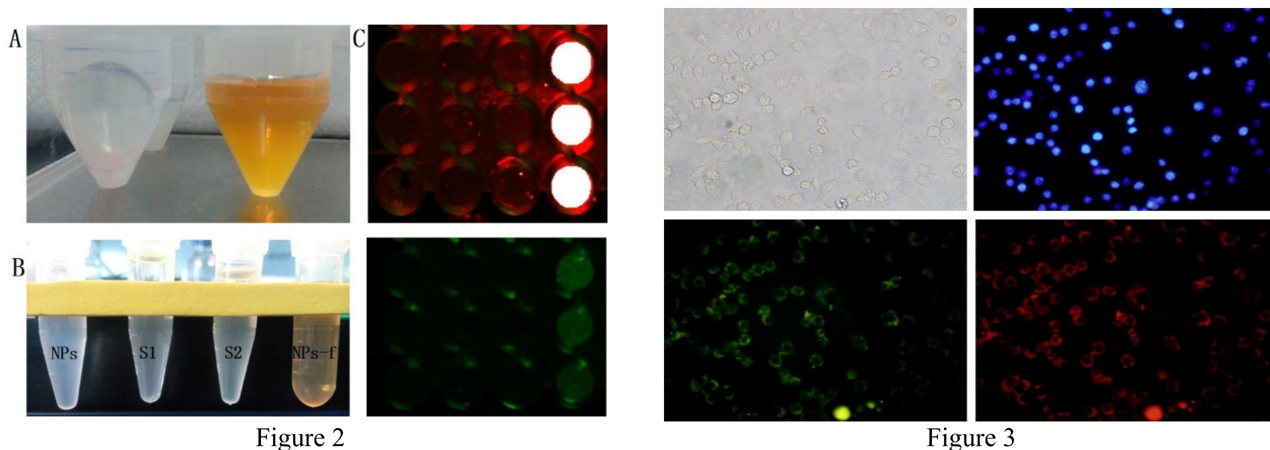

Figure 2 Figure 3

Fig.2 (A) The NPs and the NPs were treated with glutaraldehyde and glycine. (B) NPs: Untreated nanoparticles; S1: Ultra-filtered supernatant; S2: The supernatant of ethanol precipitation treatment; NPs-f: The final treated nanoparticles. (C) Appearance of NPs, S1, S2 and NPs-f solution, and NIRF imaging at 720 and 820 nm emission wavelength.
Fig.3 Treatment of RAW264.7 cells with polyacrylamide NPs for 4 h. The cell nuclei were then stained by DAPI and the cells were imaged at the bright field. Microscope images of DAPI, green fluorescence, red fluorescence were obtained, respectively.

4. Conclusion

This study developed a method for preparing autofluorescent polyacrylamide NPs, which successfully prepared polyacrylamide NPs. The nanoparticles were efficiently internalized into RAW264.7 cells and displayed strong green and red fluorescence with fluorescence microscope, respectively. Moreover, It was found that polyacrylamide NPs possessed NIR autofluorescence (NIRF) in addition to their visible fluorescence, which is critical for in vivo application of the nanomaterial, indicating a wide potential biomedical applications in future.

Acknowledgments

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