One-pot Synthesis of Multifunctional PAM@FeNPs Composite Microspheres

Jun Wang¹, and Jinke Wang¹,*

¹ State Key Laboratory of Bioelectronics, Southeast University, Nanjing, China
*Corresponding author e-mail: wangjinke@seu.edu.cn

Abstract. Multifunctional composite microspheres are with variety of applications. In this study, one-pot dot polymerization synthesis was utilized to construct the polyacrylamide@Fe₃O₄ nanoparticles (PAM@FeNPs) composite microspheres. This composite microspheres featured as uniform size, great magnetic property, extremely stable visible and near-infrared autofluorescence produced by glutaraldehyde crosslinking. In conclusion, this study provides an important insight into the preparation of multifunctional composite microspheres.

1. Introduction

Recently, the preparation of multicomponent composite biomaterials has been a promising strategy to obtain biomaterials with multifunction¹,². And organic-inorganic composite microspheres have received much interest due to their potential applications in controlled drug delivery³, tissue engineering⁴, etc. Among these materials, composite polyacrylamide microsphere (PAMMP) is an important type of materials due to its outstanding biocompatibility, water solubility, and controllable surface functional groups. There have been some research that reported the synthesis of inorganic-PAMMPs composite microspheres. For example, Ag-P(AM-co-MAA) composite microspheres with patterned surface structures has been synthesized using polymeric microgel template method⁵.

Fluorescence imaging techniques, which possess high resolution and sensitivity, are ideal technology for the disease diagnosis⁶. Fluorescence imaging techniques are usually based on inherent fluorescence molecules or a synthetic fluorescent probe. Therefore, there are increasing demand in stable, biocompatible fluorescent chemical substance or probe. Furthermore, when used as crosslinking agent, glutaraldehyde (GTA) can produce fluorescence⁷. There are several studies that reported the fluorescence nanoparticles or microspheres based on GTA crosslinking⁸,⁹. However, there is no research reporting preparation of composite microspheres with visible or near-infrared autofluorescence.

In this study, we firstly synthesized the monodisperse polyacrylamide microspheres with visible and near-infrared fluorescence (fPAMMPs) utilizing GTA crosslinking via improved reverse microemulsion polymerization, named dot polymerization. The diameter of fPAMMPs ranging from about 180 μm to 800 μm was achieved through changing the dot volume. Furthermore, the polyacrylamide-Fe₃O₄ nanoparticles (PAM@FeNPs) composite microspheres were successfully synthesized via one-pot synthesis. This multicomponent microspheres processed good magnetic property, stable visible autofluorescence, and even the near-infrared fluorescence (NIRF). This research provided a promising multifunctional composite microspheres for fluorescence optical imaging, a post-loading drug or biomacromolecule carrier.
2. Materials and Methods

2.1. Materials

N-(3-aminopropyl)methacrylamide hydrochloride (APMA) and acrylamide (AM) were obtained from Sigma Aldrich (MO, USA). Glutaraldehyde (GTA) was obtained from Sinopharm Chemical Reagent (Shanghai, China). Mineral oil, ammonium persulfate (APS), N,N,N',N'-tetramethylethane-1,2-diamine (TEMED) and N,N'-methylidenebis acrylamide (MBA) were purchased from Biosharp Biological Technology (Hefei, China). Polylysine was obtained from Shifeng (Shanghai, China). Polyethyleneimine functionalized Fe₃O₄ nanoparticles (PEI@FeNPs) and dimercaptosuccinic acid functionalized Fe₃O₄ nanoparticles (DMSA@FeNPs) were purchased from Nanoeast Biotechnology (Nanjing, China).

2.2. Preparation of fPAMMPs via dot polymerization

PAMMPs were prepared as follows: 264 mg AM, 80 mg MBA, 25 mg APMA were dissolved in 1 mL deionized (DI) water under ultrasound to obtain the uniform acrylamide monomer liquid. The mixture solution contained 80 μL acrylamide monomer liquid, 80 μL 10% ε-PL, 5 μL 20% APS. 0.5 μL aliquots of the mixture solution were pipetted on a plate with polyethylene to form dots, which were then covered by the mineral oil containing 0.4 % TEMED. Dots were polymerized at 37 °C for 1 min. After removing the mineral oil, the prepared PAMMPs were washed three times with DI water. PAMMPs with different diameters could be obtained by altering the dot volume of the mixture solution. BioDot AD1500 Aspirate/Dispense Platform was used to spot 200 nL, 100 nL, 50 nL, 30 nL, 20 nL, 10 nL aliquots of the mixture solution. 20 μL 25 % glutaraldehyde was added into PAMMPs solution and incubated at 37 °C for 30 min. The fPAMMPs were washed three times with DI water to remove excess glutaraldehyde. The same procedure was used to prepare fPAMMPs without ε-PL by using DI water to replace ε-PL.

2.3. Preparation of PAM@FeNPs composite microspheres

In order to obtain magnetic fPAMMPs, two methods were developed to synthesize PAM@FeNPs composite microspheres. One is post-loading. The prepared fPAMMPs were centrifuged to remove water and then rested for 1 h to remove the residual water as possible. The dried fPAMMPs were mixed with 100 μL of 1mg/mL DMSA@FeNPs at 37 °C overnight to obtain PAM@FeNPs composite microspheres. The composite microspheres were washed three times with DI water to remove excess FeNPs. The other is one-pot synthesis. The composition of the mixture solution containing 40 μL acrylamide monomer liquid, 40 μL 10% ε-PL, 80 μL 1 mg/mL PEI@FeNPs was incubated for 10 min and then mixed with 5 μL 20% APS. The other steps were exactly the same as the preparation of fPAMMPs.

2.4. Characterization

The morphologies and visible fluorescence were detected and imaged using an IX51 inverted fluorescence microscope. The IRDye 800CW streptavidin were post-loaded and then the NIRF at the emission wavelength of 720 and 820 nm were detected with Odyssey Infrared Imaging System.

3. Results and Discussion

3.1. Preparation and characterization of fPAMMPs

There have been various studies that reported the preparation of PAMMPs via reverse microemulsion polymerization[10, 11]. However, PAMMPs prepared by this method are generally not monodispersed because the droplets in oil phase during stirring process, which is necessary for reverse microemulsion polymerization, are difficult to be completely identical. Therefore, we prepared the PAMMPs with uniform size via dot polymerization and achieved the control of different size ranging from ~180 μm to ~800 μm via altering the dot volume (Table 1, Figure 1a). By utilizing biodot platform, about 2,000 PAMMPs could be obtained within 30 min, largely improving the productivity compared with hand-spotting.
GTA crosslinking which can produce visible fluorescence and NIRF has been used to constructed fluorescence nanoparticles. Two different double bonds, C=C and C=N, are the main source of fluorescence produced via GTA crosslinking and therefore the fluorescence property can be strengthened by increasing amino groups of nanoparticles. Herein, GTA crosslinking was used to prepare fluorescence microspheres. The results showed that fPAMMPs with ε-PL showed that visible fluorescence and NIRF were both enhanced compared with fPAMMPs without ε-PL (Figure 1b, c). Furthermore, even after being irradiated under excitation light for more than 10 minutes, visible fluorescence of fPAMMPs was not significantly reduced, demonstrating that fPAMMPs had excellent fluorescence stability. Interestingly, for visible fluorescence, green and red fluorescence are obviously stronger than blue fluorescence (Figure 1b). The molecule mechanism of this phenomenon is not clear.
and further research need to be performed. The fPAMMPs’ affinity to biomacromolecule was investigated. After incubated with IRDye 800CW streptavidin for 2 h, the NIF at the emission wavelength of 820nm was obviously enhanced (Figure 2), which indicated that streptavidin was successfully post-loaded into the fPAMMPs.

3.2. Preparation of PAM@FeNPs composite microspheres

The rational combination of the superior properties of different component provides a feasible approach to obtain multifunctional composite microspheres. At the beginning, one-pot synthesis was used to prepare the composite microspheres, but PEI@FeNPs aggregated within several minutes after mixed with APS (Figure 3a), which may be caused by the destruction of nanoparticles’ surface structure due to the existence of strong electrolyte, APS. Under this circumstance, although the composite microspheres were synthesized and still magnetic, fluorescence microscopy results showed that FeNPs are very unevenly distributed inside the microspheres and hinder the fluorescence of microspheres (Figure 3b, d). Therefore, post-loading synthesis was developed since streptavidin can be post-loaded into the fPAMMPs. The negatively charged DMSA@FeNPs was chosen because it can combine with positively charged fPAMMPs by electrostatic interaction. After overnight incubation, the magnetic composite microspheres were obtained, there was still the uneven distribution of FeNPs, even if it was lessened than before (Figure 3c, e).

Finally, one-pot synthesis was optimized to prepare PAM@FeNPs composite microspheres. We occasionally found that PEI@FeNPs didn’t aggregate if APS was added after incubation the mixture containing PEI@FeNPs and ε-PL for several minutes. Amino-rich ε-PL acted as the stabilizer to protect the surface structure of nanoparticles from APS in this process. The multifunctional PAM@FeNPs composite microspheres were successfully synthesized. 50, 100, 500nL dot volume were chosen and magnetic property, visible fluorescence and NIRF were detected. The results showed that the composite microspheres owed good magnetic properties, being absorbed onto the magnetic stand within half a minute (Figure 4a). And the microscopy observation and NIRF imaging results demonstrated that visible fluorescence and NIRF of PAM@FeNPs composite microspheres were the same as the fPAMMPs, not interfered by FeNPs (Figure 4b, c).
Figure 3. (a) Appearance of PEI@FeNPs after mixing with APS. Right is normal appearance of PEI@FeNPs. Photographs of PAM@FeNPs composite microspheres (500 nL dot volume) prepared by one-pot synthesis (b) or post-loading synthesis (c) after placing on magnetic stand. Fluorescence images of PAM@FeNPs composite microspheres (500 nL dot volume) prepared by one-pot synthesis (d) or post-loading synthesis (e). Scale bar is 500 μm.

Figure 4. a) Photographs of PAM@FeNPs composite microspheres after placing on magnetic stand within half a minute. b) NIRF images of PAM@FeNPs composite microspheres at the emission wavelength of 720 and 820 nm. c) Fluorescence images of PAM@FeNPs composite microspheres. Scale bar is 200 μm.
4. Conclusion

A unique multifunctional microspheres composed of polyacrylamide and Fe$_3$O$_4$ nanoparticles was prepared through one-pot dot polymerization. The dot polymerization ensured the uniformity of microspheres and the biodot platform significantly improved the productivity of manufacturing microspheres. Furthermore, one-pot synthesis was developed to combine the visible and near-infrared fluorescence of PAMMPs produced by GTA crosslinking and magnetic property of FeNPs together. This magnetic PAM@FeNPs composite microsphere with visible fluorescence and NIRF has huge potential application in biomedical imaging and drug delivery system, and more importantly, provides an insight into preparation of multifunctional composite microspheres. It is expected that other nanoparticles-organic composite microspheres with multifunction might be also prepared via the proposed one-pot polymerization synthesis.

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