Near-infrared Fluorescence Imaging and Optimization of Polyacrylamide

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Abstract. Polyacrylamide (PAA) is a reticular macromolecular compound, and the C=N bonds produced by the reaction with glutaraldehyde reveal intensive near-infrared fluorescence (NIRF). This paper studies the effect factors on the fluorescence intensity of PAA microspheres (beads), including the particle size of PAA beads, APMA concentrations, and different concentrations of glycine treatment by infrared imaging system and fluorescence microscope. The results demonstrate that PAA gel and PAA beads display autofluorescence at the emission wavelength of 700 nm and 800 nm， respectively. The smaller the particle size of beads is, the better the fluorescence intensity is in a certain range. The beads have stronger NIRF when 11 mg/mL APMA is in monomer liquid compared with the beads containing 7 mg/mL or 9 mg/mL APMA in monomer liquid. Glycine treatment enhances the fluorescence intensity of the beads, which is the most obvious at 0.2 M glycine in solution.

1. Introduction

Polyacrylamide (PAA) gel is a macromolecule polymerization of acrylamide and N, N'-Methylene-Bis-Acrylamide (bisacrylamide) under the action of cross-linking agent. PAA is widely applied to thickening [1], reducing drag [2], adsorption [3], flocculation [4], biomedical materials and so on for many excellent advantages, including good flocculation, low cost, easy preparation. In biomedical research, PAA materials are prepared in different forms based on various needs and have a wide range of applications. For instance, PAA gel is often used in the separation and purification of nucleic acid and proteins. PAA hydrogel has good application prospects in plastic surgery [5] and drug release because of its excellent biological compatibility, innocuity and environmental friendliness. PAA nanoparticles (PAANP) have huge application potential in drug loading [6] and tumor near-infrared fluorescence imaging [7]. PAA microspheres (PAA beads) have been applied to SNP genotyping [8] in previous studies.

Glutaraldehyde is a kind of straight chain aliphatic dialdehyde with each aldehyde group in position 1 and 5, which can react with various chemical groups respectively. Therefore, glutaraldehyde has been widely used in cell protein immobilization [9] and the crosslinking of biomaterials [10]. It has been reported that the C=N bonds on Schiff base which produced by the reaction between amino groups and aldehyde groups display autofluorescence [11]. Since the amino groups on acrylamide and bisacrylamide are rather inert, N-(3-Aminopropyl)methacrylamide hydrochloride (APMA) is employed as the comonomer to carry out the copolymerization [12] and increase the active amino groups. Glycine is a common amino acid and is often used in biochemical experiments and organic synthesis. The amino groups on glycine can react with the aldehyde groups on glutaraldehyde to produce C=N bonds, which is utilized to enhance the fluorescence intensity of the polyacrylamide in this study.

This paper studies the preparation method of fluorescent polyacrylamide materials and researches the effects of the particle size of PAA beads, APMA concentrations and different concentrations of glycine treatment on the fluorescence intensity.

2. Materials and Methods

2.1 The preparation of fluorescent PAA gel in 384 well plates and fluorescent PAA beads

Polyacrylamide monomer liquid: 264 mg Acrylamide, 80 mg N, N'-Methylene-Bis-Acrylamide, 7 mg N-(3-Aminopropyl)methacrylamide hydrochloride (APMA), 1 mL H2O, dissolution under ultrasound at 37 ℃. The gel solutions contained 1 mL monomer liquid, 985 μL H2O, 14 μL Ammonium Persulphate (APS) and 1 μL N,N,N', N'-Tetramethylethylenediamine (TEMED). The gel solutions were pipetted 20 μL into each well of 384 well plates. The gel was polymerized at 37 ℃ for 1 min. Add 20 μL glutaraldehyde (5 %) each well into 3 wells and 20 μL H2O each well into another 3 wells as a control. The reaction was at 37 ℃ overnight.

Prepare the same monomer liquid as above. The mixed solutions contained 335 μL monomer liquid, 50 μL sodium borate buffer(0.2 M), 20 μL APS, 595 μL H2O. The mineral oil containing 0.4 % TEMED was placed into a plate with polyethylene, and 1.0 μL aliquots of the mixed solution were pipetted under the mineral oil. Beads were polymerized at 37 ℃ for 1 min. The mineral oil was decanted and the beads were recovered in H2O. Half of these beads were put into 1 mL glutaraldehyde (5 %) and the other half were put into 1 mL H2O as a control. The reaction was at 37 ℃ overnight.

2.2 Different effect factors of the fluorescence intensity of the beads

The particle size of PAA beads: Make the beads as above. 1.0 μL, 0.5 μL and 0.25 μL aliquots of the mixed solution were pipetted under the mineral oil for making different particle sizes of beads. Put these three kinds of beads into different 1.5 mL centrifuge tubes and add 1 mL glutaraldehyde (5 %) separately. The reaction was at 37 ℃ overnight.

APMA concentrations: Prepare 3 kinds of different monomer liquids, containing 7 mg, 9 mg, 11 mg APMA separately. Make the beads as above. Put these three kinds of beads into different 1.5 mL centrifuge tubes and add 1 mL glutaraldehyde (5%) separately. The reaction was at 37 ℃ overnight.

Different concentrations of glycine treatment: Make the beads as above and the monomer liquid contains 11 mg APMA. Use 5 % glutaraldehyde to react with the beads at 37 ℃ overnight and wash them with water. Then these beads were soaked in 1 mL 0 M, 0.1 M, 0.2 M, 0.3 M glycine solutions separately at 37 ℃ for 6 h.

3. Results

3.1 NIRF of glutaraldehyde-treated PAA gel and PAA beads

Glutaraldehyde–treated PAA gel was imaged with various fluorescence intensity by using Odyssey Infrared Imaging System and the results indicated that the glutaraldehyde-treated PAA gel showed autofluorescence at the emission wavelength of both 700 nm and 800 nm while PAA itself did not emit fluorescence (Fig.1a).

PAA beads after treated by glutaraldehyde were also imaged under various fluorescence intensity and the results of NIRF revealed that the glutaraldehyde-treated PAA beads showed autofluorescence at the emission wavelength of both 700 nm and 800 nm but PAA itself did not emit fluorescence and the fluorescence at 700 nm was stronger than that at 800 nm (Fig.1b).

3.2 The effect factors of the fluorescence intensity of PAA beads

The imagings by the fluorescence microscope show that different particle sizes of glutaraldehyde-treated PAA beads all had intensive green, red and blue fluorescence (Fig.2a). NIRF imaging results suggest the smaller the particle size of beads is, the stronger the fluorescence intensity is in a certain range (Fig.2b).

The NIRF scanning results demonstrate that the beads containing 11 mg/mL APMA in monomer liquid had stronger fluorescence no matter at the emission wavelength of 700 nm or 800 nm

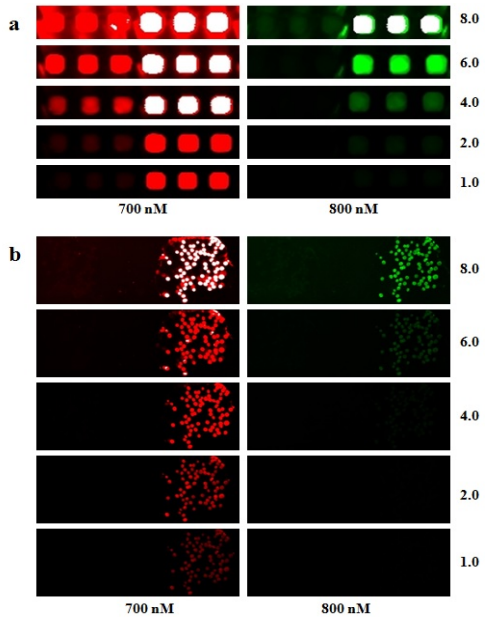

Fig. 1. Near infrared fluorescence (NIRF) of glutaraldehyde-treated PAA. (a) NIRF of glutaraldehyde-treated PAA gel. (b) NIRF of glutaraldehyde-treated PAA beads.

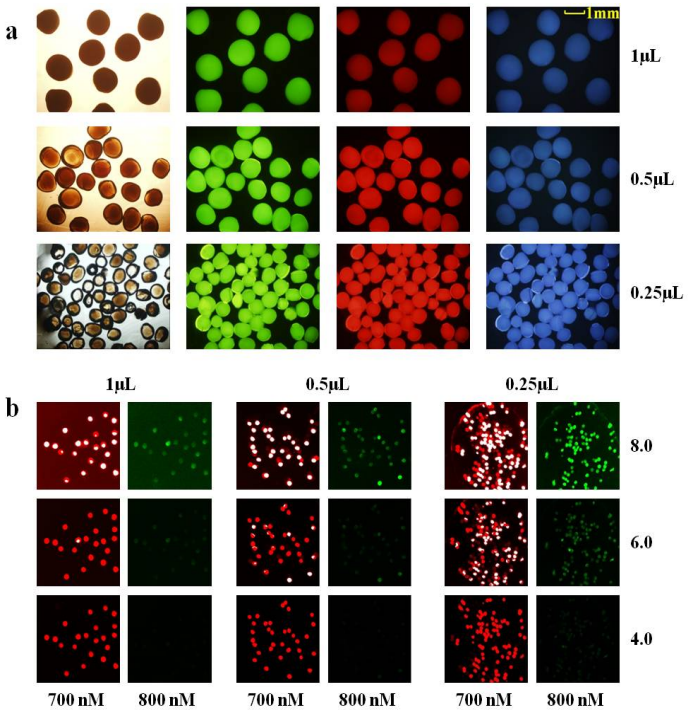

Fig. 2. NIRF of glutaraldehyde-treated different particle sizes of PAA beads. (a) Beads imaging with fluorescence microscope. Beads were imaged at the bright field and three different fluorescence channels, green, red and blue fluorescence. Magnification: 10 × 4. (b) NIRF of different particle sizes of PAA beads at the emission wavelength of both 700 nm and 800 nm.

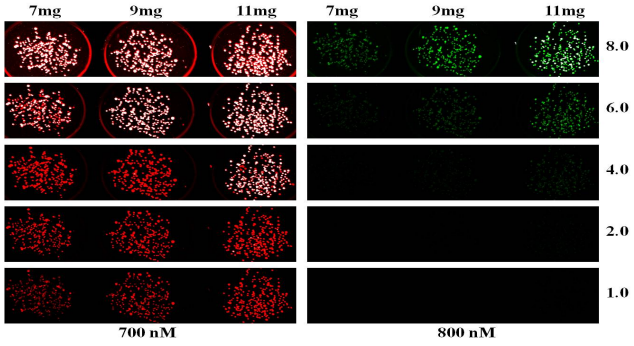

Fig. 3. NIRF of glutaraldehyde-treated PAA beads containing different concentrations of APMA.

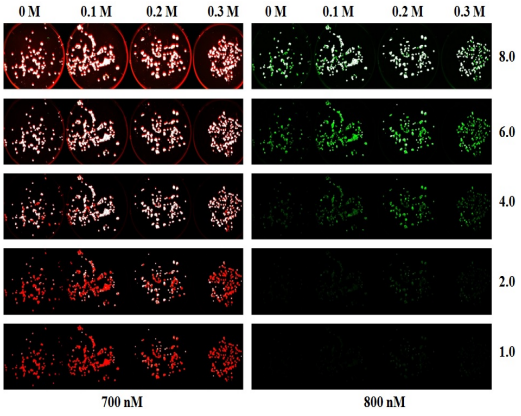

Fig. 4. NIRF of different concentrations of glycine treatment on PAA beads after treated by glutaraldehyde.

compared with the beads containing 7 mg/mL or 9 mg/mL APMA in monomer liquid (Fig.3), indicating that the higher the concentration of APMA in beads, the more beneficial it can be to the enhancement of the fluorescence intensity to a certain extent.

Because of the excess of glutaraldehyde in the reaction of polyacrylamide and glutaraldehyde, when beads is soaked in glycine solution, the amino groups on the glycine can continue to react with excess aldehyde on the beads to produce C=N bonds in order to enhance the fluorescence intensity. The NIRF imaging results revealed that when glycine solution is 0.2 M, the enhancement effect of fluorescence intensity is most obvious (Fig.4).

4. Summary

This paper studied the preparation of fluorescent PAA gel and fluorescent PAA beads on the basis of the autofluorescence at the emission wavelength of both 700 nm and 800 nm produced by the reaction between the amino groups and the aldehyde groups. The NIRF imaging results indicate that smaller particle size of PAA beads and higher concentration APMA had stronger fluorescence in a certain range. The fluorescence intensity was significantly enhanced in 0.2 M glycine solution. The most suitable size of PAA beads and the optimum concentration of APMA might be explored based on different applications in further.

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