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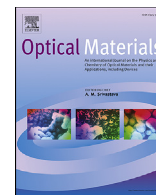

Breast cancer cells synchronous labeling and separation based on aptamer and fluorescence-magnetic silica nanoparticles

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ABSTRACT

In this work, an efficient method based on biotin-labeled aptamer and streptavidin-conjugated fluorescence-magnetic silica nanoprobes (FITC@Fe_3O_4@SiNPs-SA) has been established for human breast carcinoma MCF-7 cells synchronous labeling and separation. Carboxyl-modified fluorescence-magnetic silica nanoparticles (FITC@Fe_3O_4@SiNPs-COOH) were first synthesized using the Stöber method. Streptavidin (SA) was then conjugated to the surface of FITC@Fe_3O_4@SiNPs-COOH. The MCF-7 cell suspension was incubated with biotin-labeled MUC-1 aptamer. After centrifugation and washing, the cells were then treated with FITC@Fe_3O_4@SiNPs-SA. Afterwards, the mixtures were separated by a magnet. The cell-probe conjugates were then imaged using fluorescent microscopy. The results show that the MUC-1 aptamer could recognize and bind to the targeted cells with high affinity and specificity, indicating the prepared FITC@Fe_3O_4@SiNPs-SA with great photostability and superparamagnetism could be applied effectively in labeling and separation for MCF-7 cell in suspension synchronously. In addition, the feasibility of MCF-7 cells detection in peripheral blood was assessed. The results indicate that the method above is also applicable for cancer cells synchronous labeling and separation in complex biological system.

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1. Introduction

Breast cancer is one of the commonest malignant tumors in women. According to the statistics, there are about 1.2 million new patients with breast cancer each year in the world, of which 0.5 million patients died of breast cancer. Tumor metastasis is the leading cause of death in patients. Circulating tumor cell (CTC) is a kind of tumor cell that exists in peripheral blood, which spreads to other organs of the body by the blood circulation [1]. However, most of CTCs in peripheral blood suffer from apoptosis or cytophagy. Very few of CTCs escape and develop into metastatic lesion, and increase the mortality risk of patients. Therefore, the early detection of breast cancer CTC in the blood would play important guiding roles in the terms of monitoring of tumor recurrence, therapeutic evaluation, and individual treatment.

At present, CTCs are mainly detected using immunocytochemistry (ICC), Flow cytometry (FCM), and RT-PCR [2–4]. Briefly, ICC reflects the existence and distribution of biomacromolecules such as proteins, membrane surface antigens, and receptors, but the procedure is tedious and time-consuming. In addition, the poor photostability of fluorescein may influence the observation results. Both FCM and RT-PCR are sensitive and fast, which can quantitatively analyze the cell components such as DNA. However, cell morphology and more biological information can't be acquired by the above methods. How to develop an efficient and sensitive method for CTCs detection in complex biological system is essential in bioanalysis. With the development of nanotechnology in recent years, some fluorescent nanomaterials such as quantum dots, carbon dots, and dye-doped silica nanoparticles have showed great advantages in the field of biomedical imaging because of their unique optical properties [5–7]. In addition, the AgInZnS nanoparticles used for cell labeling is also reported [8]. However, faced with the complex system, these materials only play a role of single labeling. Other substances in the mixtures may reduce the labeling efficiency of targets and disturb the signals of detection. To enhance the sensitivity, the process of pretreatment and purification is necessary for most fluorescent-labeling based methods. In addition, although magnetic nanomaterials such as α-Fe, Fe_3O_4, and α-Fe_2O_3 have aroused great concern in the field of separation science

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[9–11], they mainly provide single separation. Besides, some magnetic materials are prone to aggregation after the removal of external magnetic field due to the hysteresis, which results in poor separation of targets. Moreover, the complex procedure of preparing biomolecules-functionalized magnetic nanoprobes and poor biocompatibility of bare magnetic nanomaterials should be considered [12]. Therefore, it is urgent to prepare composite nanomaterials with fluorescence, superparamagnetism, and good biocompatibility for targets synchronous labeling and separation.

Composite silica nanomaterials have attracted considerable attention in biomedical analysis because of their anti-photobleaching, larger specific surface area, small size, good biocompatibility, facile surface modification, and special magnetic properties [13–15]. Especially, dye-doped silica nanoparticles have been widely used for cancer cells labeling, DNA microanalysis, and the detection of pathogenic bacteria [16–18]. In addition, Fe_3O_4-enwrapped silica nanoparticles ($Fe_3O_4@SiO_2$) with superparamagnetism could be aggregated immediately in the presence of external magnetic field and dispersed rapidly in solution after the removal of external magnetic field due to no hysteresis, which have been widely used for separation, and magnetic resonance imaging (MRI) [19–21]. In this paper, to prepare composite silica nanoprobes with fluorescence and magnetism for targets synchronous labeling and separation, Fe_3O_4 nanoparticles and fluorescent dyes (FITC) are enwrapped simultaneously into the silica networks using the Stöber method [22,23]. Targeted nanoprobes are then prepared by conjugating the recognizable biomolecules to the surface of fluorescence-magnetic silica nanoparticles.

As we know, antibodies as the conventional recognition elements have been widely used for biolabeling. However, they can't meet the demands of modern biological analysis increasingly. Exploring promising recognition elements has always been a goal pursued by researchers. In recent years, the aptamer based on the systematic evolution of ligands by exponential enrichment (SELEX) has attracted great concern [24]. Aptamers are single-stranded nucleic with three-dimensional structure, which show high affinity and specificity with all kinds of targets such as cancer cells, pathogenic bacteria, Virus, ATP, and even metal ions [25–29]. Compared with antibodies, aptamers take advantages of strong tissue permeability, ignorable immunogenicity, high affinity and specificity with flexible targets, easy preparation and preservation, and small molecular weight. Inspired by the extraordinary properties of aptamer and fluorescence-magnetic silica nanoparticles, we develop an efficient and sensitive method based on biotin-labeled aptamer and streptavidin-conjugated fluorescence-magnetic silica nanoprobes for breast cancer cells in the blood synchronous labeling and separation. This technology not only sensitively, conveniently, real-timely, and dynamically indicates the tumor progression and molecular biology information but also plays important guiding roles in the terms of monitoring of tumor recurrence, therapeutic evaluation, and individual treatment.

2. Experimental

2.1. Chemicals and materials

TritonX-100, tetraethyl orthosilicate (TEOS), (3-aminopropyl) triethoxysilane (APTES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimid ehydrochloride (EDC), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), Fluorescein isothiocyanate (FITC), and Streptavidin were purchased from Sigma Chemical Co. (St. Louis. MO). Ammonium hydroxide (25-28 wt%), Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), Ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), alcohol, N,-N-Dimethylformamide (DMF), and Succinic anhydride were purchased from China Pharmaceutical Group Shanghai Chemical Reagent Co., Ltd. Human breast carcinoma MCF-7 cells lines were obtained from china center for type culture collection. FITC-labeled MUC-1 aptamer was synthesized from Beijing Biosynthesis Biotechnology Co.,Ltd. The biotin-labeled MUC-1 aptamer, and the biotin-labeled random ssDNA were synthesized in Shanghai Sangon Biological Engineering Technology & Services Co. (China), and the sequence as follows:

5′-biotin-$(CH_2)_6$-GGGAGACAAGAATAAACGCTCAAGCAGTTGATCCTTTGGATACCCTGGTTCGACAGGAGGCTCACAACAGGC-3′

5′-biotin-$(CH_2)_6$-ACAGGAGGCTCACAAACGCTCAAACAGGAGGCTCACTAACGGACGTTCGACAGGAGGCTGGACGTTCGACGA-3′

2.2. Main instrumentation

The size and uniformity of synthesized nanoparticles were measured by means of transmission electron microscope (JEOL, JEM100CXII, Japan). Photoluminescence was measured using fluorescence spectrophotometer (F96PRO, China). Fluorescence images results were observed with under inverted fluorescence microscope (Nikon ECLIPSE TE2000-U, Japan). Magnetic characterization of synthesized nanoparticles was performed using vibrating sample magnetometer (Lakeshore 7410, USA). Structural analysis of prepared nanoprobe was performed using X-ray diffraction (XRD-6000, SHIMADZU).

2.3. Fe_3O_4 nanoparticle synthesis

Fe_3O_4 nanoparticles were prepared by the coprecipitation method. Briefly, 6.76 g of $FeCl_3 \cdot 6H_2O$, 2.49 g of $FeCl_2 \cdot 4H_2O$, and 200 mL of deionized water were added together to a flask. 0.4 M ammonia hydroxide was then gradually added to the iron chloride solution stirred at 350 rpm using a mechanical stirrer until pH reached 10.0. The reaction was allowed to continue for 30 min at 30 °C, followed by centrifuging and washing with deionized water three times. The products were finally resuspended in 95% ethanol for the next step.

2.4. Preparation of FITC-APTES precursor

35 μL of APTES and 3 mg of FITC were mixed in 0.5 mL of absolute ethanol under dry nitrogen atmosphere and stirring magnetically for 12 h. In addition, the FITC-APTES conjugates solution was protected from light during reaction and storage.

2.5. Preparation of carboxyl-modified fluorescent-magnetic silica nanoparticles

Carboxyl-modified fluorescence-magnetic silica nanoparticles ($FITC@Fe_3O_4@SiNPs$-COOH) were synthesized using the Stöber method. Briefly, 40 mL of ethanol, 10 mL of deionized water, and 0.5 mL of 50 mg/mL Fe_3O_4 were sonicated for 90 min and then stirred at 350 rpm using a mechanical stirrer. 1 mL of ammonia hydroxide and 200 μL of FITC-APTES were then added to the solution stirred. To create the silica coating, 1 mL of TEOS was added, and the mixture was stirred for 4 h to complete the hydrolysis process. For postcoating, 50 μL of TEOS and 50 μL of APTES were added and additional stir was performed for 2 h. By washing and resuspending with DMF solution, the products were reacted with succinic anhydride under nitrogen gas for 24 h with continuous stirring. Then the prepared $FITC@Fe_3O_4@SiNPs$-COOH were washed by water and resuspended in PBS for the next step.

2.6. Conjugation of streptavidin onto $FITC@Fe_3O_4@SiNPs$-COOH

0.5 mL of $FITC@Fe_3O_4@SiNPs$-COOH, 1 mg of EDC, and 2.5 mg of

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