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Anti-Her-2 monoclonal antibody conjugated polymer fluorescent nanoparticles probe for ovarian cancer imaging

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ABSTRACT

Fluorescent nanoparticles (FNPs) with unique optical properties may be useful as biosensors in living cancer cell imaging and cancer targeting. A novel kind of polymer fluorescent nanoparticles (PFNPs) was synthesized and its application for ovarian cancer imaging with fluorescence microscopy imaging technology was presented in this study. The PFNPs were synthesized with precipitation polymerization by using methacrylic acid (MAA) as monomer, trimethylolpropane trimethacrylate (Trim) as cross-linker, azobisisobutyronitrile (AIBN) as radical initiator and butyl rhodamine B (BTRB) as fluorescent dye. And the fluorescent dye was embedded into the three-dimensional network of the polymer when the polymer was produced. With this method the PFNPs can be prepared easily. And then the PFNPs were successfully modified with anti-Her-2 monoclonal antibody. The fluorescence probe based on anti-Her-2 monoclonal antibody conjugated PFNPs has been used to detect ovarian cancer cells with fluorescence microscopy imaging technology. The experimental results demonstrate that the anti-Her-2 monoclonal antibody conjugated PFNPs can effectively recognize ovarian cancer cells and exhibit good sensitivity and exceptional photostability, which would provide a novel way for the diagnosis and curative effect observation of ovarian cancer cells.

1. Introduction

Ovarian cancer is the leading cause of death from gynecologic cancer in the world [1–5]. It is the fifth leading cause of cancer death for women after lung, breast, colorectal and pancreatic cancer. In 2007, 22,430 new cases of ovarian cancer were diagnosed, accounting for approximately 3% of all the cancers in women and about 15,280 women will die this year because of the disease in the US [6]. Because there are few warning signs or symptoms, and malignant cells can escape from the ovarian capsule and disseminate throughout the peritoneal cavity, early detection of ovarian cancer is very difficult [7–12].

Early cancer diagnosis, in combination with the precise cancer therapies could eventually save millions of lives. Over the last 70 years, despite tremendous advances in our understanding of the molecular and cellular processes of cancer, there has been no change in the age-adjusted mortality due to cancer [13]. In order to further reduce the morbidity and mortality due to cancer, the diagnosis of cancer at the early stage is extremely challenging and has been an active research area these days.

Bio-labeling with fluorescent dyes has many applications in biomedical science, especially in the field of cancer imaging and cancer targeting. Fluorescence microscopy is among

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the most widely used approaches for high resolution, noninvasive imaging of live organisms and organic fluorophores are the most commonly used tags for fluorescence-based imaging. Despite their considerable advantages in live cell imaging, organic fluorophores suffer from the disadvantages like the inefficiency of labeling, lacking of photostability in addition to the problem of relatively low fluorescence intensity of dyes. In addition to small organic fluorophores, semiconductor nanocrystals (quantum dots, QDs) and fluorescent nanoparticles (FNPs) represent a promising new generation fluorescent label, owing to their inherent advantages. Several cancer cells were detected by using QDs as fluorescent label in the issued papers [14–21]. However, the QDs were difficult to synthesize for the rigor experimental conditions, such as high pressure, high temperature, free of water and so on. Furthermore, the application of QDs has been limited by their unbiocompatibility because of their poor solubility in water (unless they are modified) and agglutination as optical probes [22].

In the past few years, several kinds of FNPs including the dye-doped silica nanoparticles were widely studied [23–31]. Using this kind of FNPs, the feasibility of developing optical imaging technique for the sensitive detection of cancer has been recently demonstrated [32–41]. However, the dye leakage from the matrix in the dye-doped nanoparticles is still a problem no matter which kind of matrix was used. In order to solve this problem, lots of methods have been employed in the issued papers. Conjugating dye to nanoparticles, changing dye solubility, increasing electrostatic attraction force between nanoparticles and dye, increasing dye size are all usually used methods [26,42]. To a certain extent, these methods could decrease the leaking of the dye from the nanoparticles, but most of the methods have their intrinsic disadvantages. For example, it is very difficult to conjugate dye to nanoparticles if the dye has no active group.

In this study, a new type of polymer fluorescent nanoparticles (PFNPs) has been developed with precipitation polymerization method. This kind of PFNPs shows the advantages of excellent photostability, low leakage and uniform particles size. And the PFNPs were successfully modified with anti-Her-2 monoclonal antibody. This kind of fluorescence probe based on the anti-Her-2 monoclonal antibody conjugated PFNPs has been used to detect ovarian cancer cells with fluorescence microscopy imaging technology.

2. Experimental

2.1. Materials

Methacrylic acid (MAA), acetonitrile, KH_2PO_4, and Na_2HPO_4 were purchased from Xi'an Chemical Reagent Company (Xi'an, China). Butyl rhodamine B (BTRB) and azobisisobutyronitrile (AIBN) were obtained from Shanghai Chemical Plant (Shanghai, China) and used as supplied. Trimethylolpropane trimethacrylate (Trim) was from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). *N*-Hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) were obtained from Acros (New Jersey, USA). Anti-Her-2 monoclonal antibody was obtained from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). RPMI-1640 medium was purchased from HyClone Biochemical Product Co., Ltd. (Beijing, China). Unless otherwise stated, all chemicals and reagents used in this study were of analytical grade quality.

2.2. Synthesis of polymer fluorescent nanoparticles

PFNPs were synthesized by using precipitation polymerization method described as follows. The dye BTRB, MAA (0.8 mmol), acetonitrile (40 mL) and Trim (0.48 mmol) were placed into a 100 mL glass tube and the mixture was sonicated for about 10 min. Subsequently, AIBN (3 mg) were added. Then the mixture was purged with N_2 for 10 min. The glass tube was sealed and thermostated at 58 °C to start the polymerization process. After 12 h, the production was collected by centrifugation, successively washed three times with methanol and once with acetonitrile. The obtained nanoparticles were air dried. The synthesized PFNPs were characterized by spectrofluorophotometer for fluorescence intensity and by transmission electron microscope (TEM; Hitachi H700) for the morphology. The size and size distribution of PFNPs dispersed in water were measured by particle size analyzer (PSA, BI-90Plus, Brookhaven Instruments. Corp.).

2.3. Test of photostability

To investigate whether dye BTRB molecules is photostable when embedded in polymer nanoparticles, the intensity of the fluorescence was monitored vs. time [22,43]. Measurements were performed in solution of PFNPs or free dye. The solutions were continuously illuminated by the 150 W Xenon lamp at their optimal excitation wavelengths for 60 min using spectrofluorophotometer (Shimadzu RF-540). And the fluorescence intensity was acquired every 2 min over a 60 min period. A leaching test was performed as described with Wang and co-workers [44] by 1 mg mL^{-1} nanoparticles solution and monitoring the fluorescence of solution of PFNPs over 24 h.

2.4. Covalent immobilization of the anti-Her-2 monoclonal antibody onto polymer fluorescent nanoparticles surface

The anti-Her-2 monoclonal antibody was directly immobilized onto the PFNPs with well-established carbodiimide method. The immobilization protocols were the following: (1) 2 mg of PFNPs was dispersed into 1.0 mL PBS buffer; (2) 10 mg EDAC and 1 mg NHS in 1.0 mL water were added and allow the mixture to react for 15 min at room temperature with continuous mixing. (3) After the nanoparticles were washed twice with PBS, 2 mg anti-Her-2 monoclonal antibody dissolved into 0.5 mL PBS was added and allowed reacting at room temperature for 4 h with constant mixing. (4) The production, namely, anti-Her-2 monoclonal antibody conjugated PFNPs were washed with PBS several times and kept at 4 °C in PBS.

2.5. Cell culture

Human ovarian cancer cells (SKOV-3) were obtained from Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). SKOV-3 cell was routinely maintained in RPMI-1640 medium containing 10% FBS (fetal serum bovine) at 37 °C in