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Anti-epithelial cell adhesion molecule monoclonal antibody conjugated fluorescent nanoparticle biosensor for sensitive detection of colon cancer cells

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ABSTRACT

In this paper, a sensitive and selective sensor for detecting colon cancer cells based on nanoparticle covalent modified anti-human epithelial cell adhesion molecule (EpCAM) antibody is developed. The transmission electron microscope (TEM) images showed that the nanoparticle and functionalized nanoparticle had good decentrality for application. The $NaIO_4$ oxidation method, which was used as oxidizing antibody for immobilization of conjugating antibody on the silica-coated fluorescent nanoparticles, maintained the activities of antibodies very well. The fluorescence microscopy imaging and flow cytometer (FCM) experiments demonstrated that the nanosensor could increase the signal intensity obviously and distinguish three kinds of target cells (colo205, sw480 and NCM460) well. The membrane and nuclear staining showed the distribution and abundance of EpCAM in cells' membrane. It also provides a possibility to quantify special membrane proteins on different regions of cells' surface. At the end, the result of detecting a simple sample proved that colo205 cells were selected by anti-EpCAM antibody nanosensors in this environment, and made a good foundation for subsequent research.

1. Introduction

Colon cancer is the common type of colorectal cancer which includes cancerous growths in colon, rectum and appendix. It occurs more frequently than rectal cancer. The ratio of colon to rectum in developed countries is similar with developing countries, which both are 2:1 or more. There are about 9% of all malignancies in new colon cases diagnosed each year. The high risk areas include North America, Europe and Australia. Central and South America, Asia and Africa are areas of lower risk (Labianca et al., 2010). Although most patients present with surgically resectable disease, a majority of the patients will die from advanced colorectal cancer despite subsequent radiation or chemotherapy (Blum, 1995). Therefore, colon cancer is a significant public health problem.

Colon cancer etiology is characterized by a complex combination of genetic and epigenetic events (Hollande et al., 2010). Its tumorigenesis is driven by the accumulation of a limited number of genetic alterations in oncogenes and tumor suppressor genes (Pinedaa et al., 2010). There is evidence that environmental factors contribute to the development of this cancer (Kannan et al., 2008). Colon cancer does not arise de novo, but rather is preceded by histologic progression from a normal appearing mucosa at risk for colorectal neoplasia to benign neoplastic tubular and villous adenomas to carcinoma formation (Kronborg and Fenger, 1999).

The main clinical diagnostic strategies of colon cancer are biopsy, endoscopy, radiological techniques and serological markers. These methods have idiographic advantages in some situations. However, they all cannot achieve the desire of early diagnosis. Primary diagnosis of cancer cells requires sensitive and specific analytical methods. Nucleic acid-based methodology and immunocytochemical techniques have been in vogue. The original nucleic acid-based methodology was detection of DNA (Mancuso and Sternberg, 2005; Pickhardt et al., 2003; Cotton et al., 2004; Luboldt et al., 2000; Hartmann et al., 2006; Wang et al., 2009; Boni et al., 2007; Lecomte et al., 2010). The major disadvantage of circulating DNA material is that it can be released by dying tumor cells and remains in the circulation because of its relatively high stability in comparison to RNA. Therefore, the detection of RNA is the central content of nucleic acid-based methodology (Fletcher, 1986; Eisenberg et al., 1982; Newland et al., 1981; Olson et al., 1980; Zinkin, 1983; Dershaw et al., 1990). Because of the advantages of identification and imaging in cancer cell studies, immunocytochemical techniques have been paid much attention in recent years. Epithelial cell adhesion molecule (EpCAM) was a recognized

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biomarker in detection of cancer cells using immunocytochemical techniques.

EpCAM, a type transmembrane glycoprotein with molecular weight of 40 kDa, serves important roles in cell adhesion, migration and proliferation. The expression of EpCAM is restricted to simple epithelia in normal tissues. However, in pathological situations, EpCAM can be expressed in a variety of tumor cells. EpCAM has been displayed as a surface marker of some kind of cancer stem cells as well as normal stem cells. The expression pattern and level of EpCAM change notably during the course of carcinogenesis, inflammatory diseases and even in embryogenesis. What is more, the changes of expression pattern and level of this molecule in carcinoma correlate with the changes in survival rate of patients with some tumors. Moreover, EpCAM-directed therapeutics is one of hotspots for cancer treatment by therapeutic antibodies. Thus, EpCAM is a good kind of recognition molecules for some certain carcinoma cells.

Cohen et al. (2006) evaluated the ability of immunomagnetic separation to isolate circulating tumor cells and of the fluorescent microscope system and flow cytometry to enumerate and characterize circulating tumor cells from patients with metastatic colorectal cancer. Karlsson et al. (2008) explored the potential of flow cytometry to become a fast, sensitive and reliable method for detection of lymphatic metastases in patients with colon cancer using direct fluorophore-conjugated antibodies against multiple surface antigens. Königsberg et al. (2010) compared four manual cytometric methods to detect circulating tumor cells in vitro. The EpCAM-based technology showed a significantly better tumor cell recovery rate compared to other cytometric methods. Recently, Yang et al. (2009) used different decomposition rates of fluorescent protoporphyrin IX (PpIX) in cancer cells and normal cells for detecting Caco-2 cells. PpIX degrades more slowly in Caco-2 colon cancer cells than in normal cells and can be used as a source of fluorescence to detect Caco-2. This method introduced a new idea in the detection of cancer cells.

In previous studies, three basal methods were introduced in modification of fluorescent core–shell silica nanoparticles, which were the glutaraldehyde method, EDAC method and Cyanogen bromide method. Much amelioration was also based on these methods (Peng et al., 2007; Shi et al., 2007). The common ground of these methods is that cross linker was used widely for covalent immobilization of the antibody conjugated to biomarkers. The advantages of these methods are that they are simple, fast, and easy to perform. However, the disadvantages include low immobilization efficiency, decrease of antibody activity, uncertain reaction ratio, and toxicity of the cross linkers. These disadvantages weakened capture capability of antibodies observably. In order to resolve this problem, we introduced the $NaIO_4$ oxidation method, a seldom used method for immobilization of anti-EpCAM monoclonal antibody. This method does not use the cross linker, which avoids its negative effects. Oxidation of antibodies has been shown to maintain the activity of antibodies effectively under the right conditions (Abraham et al., 1991).

We report a novel technology to detect colon cancer colo205 cells using fluorescence imaging and FCM. Anti-EpCAM antibody combined with Rubpy-encapsulated core–shell silica nanoparticles as biomarkers. The nanoparticle labels contributed to the design of bioanalysis with many advantages, such as lower detection limits, low consumption of reagents, and excellent sensitivity. The specific monoclonal antibodies were directed against membrane antigens EpCAM which is overexpressed in colon cancer colo205 cell. We also compared the conventional glutaraldehyde method with the $NaIO_4$ oxidation method for functionalizing fluorescent core–shell nanoparticles. The results show that the $NaIO_4$ oxidation method which did not use cross-linker could maintain the activity of the monoclonal antibodies more easily than the glutaraldehyde method. The results suggested that this nanosensor detects the abundance and distribution of EpCAM in cell membranes, which is helpful for the detection and characterization of cancer cells.

2. Experimental

2.1. Materials

Anti-EpCAM monoclonal antibodies, Colo205, sw480 and NCM460 were prepared by Department of Immunology (The Fourth Military Medical University, China). $Ru(bpy)_3Cl_2$ (Rubpy), Triton X-100 (TX-100), and Hoechst 33258 were obtained from Sigma (USA). (3-Aminopropy1) triethoxysilane (APS) was obtained from Acros (Belgium). HAc, NaAc, NaCl, Na_2HPO_4, KH_2PO_4, KCl, NaH_2PO_4 and $NH_3{\cdot}H_2O$ (28–30 wt.%) were purchased from Xi'an Chemical Reagent Company (Xi'an, China). Tetraethyl orthosilicate (TEOS), *n*-hexanol, $NaIO_4$, and cyclohexane were obtained from Shanghai Chemical Plant (Shanghai, China). Double distilled water was used for the preparation of all aqueous solutions.

0.15 mol/L pH7.1 PBS buffer was prepared by dissolving 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4, 8.0 g NaCl and 0.2 g KCl in 1 L water.

2.2. Preparation of Rubpy-encapsulated core–shell silica nanoparticles

The W/O microemulsion was prepared at room temperature first by mixing 1.77 mL of surfactant TX-100, 7.5 mL of oil phase cyclohexane and 1.8 mL of cosurfactant *n*-hexanol. 0.48 mL of Rubpy dye solution was then added. Then the resulting mixtures were homogenized with magnetic force stirring to form a water-in-oil microemulsion. In the presence of 100 μL of TEOS, a hydrolyzation reaction was initiated by adding 60 μL of $NH_3{\cdot}H_2O$ under stirring. The reaction was allowed to stir for 12 h. Then, 100 μL of APS and 60 μL of $NH_3{\cdot}H_2O$ were added to the mixture under stirring. The reaction was allowed to stir for another 12 h. After the reaction was completed, acetone was added to break the microemulsion and recover the particles. The nanoparticles were washed with ethanol several times to remove surfactant molecules and physically adsorbed Rubpy dye from the particles' surface. The particles were stored in ethanol. The free nanoparticles and functionalized nanoparticles were constituted about 1‰ dispersion using pH 7.1 and pH 7.6–7.8 ammonia water solution and characterized by transmission electron microscope (TEM; Hitachi H7000) for size and morphology.

2.3. Covalent immobilization of anti-EpCAM monoclonal antibodies onto core–shell silica nanoparticles by the glutaraldehyde method

(1) Wash 2 mg of nanoparticles three times in 5 mL of PBS solution to remove ethanol.
(2) After wash, resuspend nanoparticles in 5 mL of glutaraldehyde solution (final concentration of 2.5% in PBS), ensuring that the nanoparticles are completely suspended. Sonication should aid in suspension.
(3) Allow to react for 2 h at room temperature (18–25 °C), with continuous mixing.
(4) Wash two times, resuspend nanoparticles in 600 μL of PBS, and ensure that the nanoparticles are completely resuspended, as in Step 2.
(5) Add 400 μg of anti-EpCAM monoclonal antibodies to nanoparticles suspension, and react for 30 min at 4 °C with continuous mixing.
(6) Adjust pH to 7.6–7.8, and react at 4 °C with continuous mixing for 20 h.
(7) Stored at 4 °C in PBS (pH 7.6–7.8).

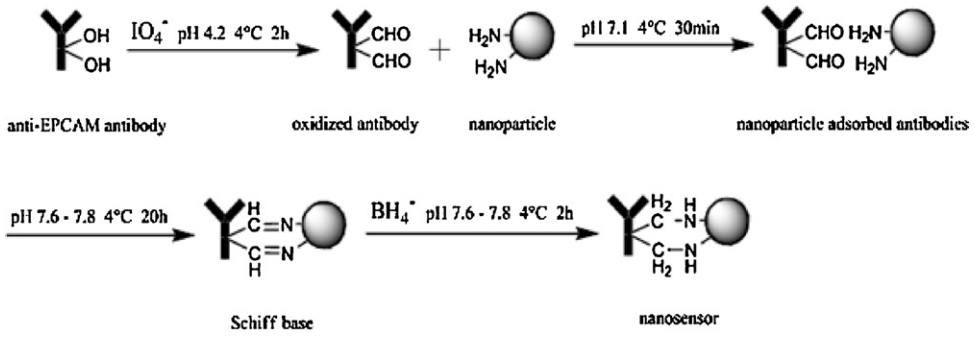

Fig. 1. Schematic illustration of the $NaIO_4$ oxidation method.

2.4. Covalent immobilization of anti-EpCAM monoclonal antibodies onto core–shell silica nanoparticles by the $NaIO_4$ oxidation method

The schematic illustration of the $NaIO_4$ oxidation method is shown in Fig. 1. The operating procedures were the following:

(A) *Oxidation of antibody* (1) dissolve 400 μg of anti-EpCAM monoclonal antibody in 70 μL of acetate buffer (0.05 mol/L, pH 4.2). (2) Add the antibody solution to 140 μL of $NaIO_4$ solution (1.5 mg/mL, pH 4.2). (3) Incubate the sample for 2 h at 4 °C in the dark with constant mixing. (4) Stop the reaction and remove unreacted $NaIO_4$ by ultrafilter, wash with the acetate buffer.

(B) *Coupling to nanoparticles* (1) wash 2 mg of nanoparticles three times in 5 mL of PBS solution in order to remove ethanol. (2) After third wash, resuspend nanoparticles in 600 μL of PBS, ensuring that the nanoparticles are completely suspended. (3) Add the oxidized antibody to nanoparticles suspension gradually, and react with constant mixing at 4 °C for 30 min. (4) Adjust pH to 7.6–7.8, and react at 4 °C with continuous mixing for 20 h. (5) Wash, resuspended the nanoparticles in 600 μL of PBS. (6) Add 20 μL of $NaBH_4$ solution (2 mg/mL, pH 7.6–7.8), and react at 4 °C for 2 h in the dark with constant mixing. (7) Wash three times, then store at 4 °C in PBS (pH 7.6–7.8) until used.

2.5. Cell culture

Colo205 (positive) cells and sw480 (negative) cells were both human colon cancer cells. NCM460 cells (negative) are normal human colon cells. After careful recovery of frozen cells, the cells were routinely maintained in a RPMI-1640 medium containing 10% FBS (fetal serum bovine) at 37 °C in a humidified 5% CO_2 – 95% air atmosphere.

2.6. Immunofluorescence and nuclear staining

Before staining, the cells need to be observed for good state. The immunofluorescence staining of cells are as follows: (1) the amounts of cells suspension were adjusted to $2 \times 10^5 - 1 \times 10^6$/mL. (2) Wash cells in PBS twice (3 min every time at room temperature) and resuspend in 100 μL PBS. (3) Add in 1 μL of nanosensor suspension, reacting in 4 °C for 40 min. (4) Wash three times in PBS for removing unreacted nanosensors.

The nuclear staining of cells is as follows: (1) Hoechst 33258 stock solution is prepared at 1 mg/mL in distilled water and stored in −20 °C. (2) Wash cells in PBS twice (3 min every time at room temperature). (3) Dilute the Hoechst 33258 stock solution to 1 μg/mL (final concentration) with PBS and suspend cells in it for 10 min at room temperature. (4) Wash cells in PBS twice. (5) Suspend cells in 20% glycerol solution. (6) Place a drop of cell suspension on a clean glass slide, squash under a cover glass. (7) Fluorescence microscope is used to detect nucleic staining (excitation wavelength 350 nm, emitting wavelength 460 nm).

2.7. Fluorescence microscopy imaging and FCM analysis

Fluorescence microscopy imaging was performed in Olympus inverted microscope system (Olympus, Model IX70, Tokyo, Japan) with a 100 W high-pressure mercury lamp (Olympus, Model BH2-RFL-T3, Tokyo, Japan) and CCD (Olympus, Model DP70, Tokyo, Japan).

The flow cytometer (FCM) detection was performed in FACSCalibur (Becton Dickson, NJ, USA).

3. Results and discussion

3.1. Characterization of nanoparticles

The inverse microemulsion method yielded uniform functionalized fluorescent core–shell nanoparticles. These nanoparticles were characterized using microscopic methods. The results showed that the particle sizes of nanoparticles were about 50 ± 5 nm (Fig. 2). We can see in Fig. 2 that free nanoparticles and nanoparticles conjugated anti-EpCAM antibodies all have good decentrality. In TEM experiment, the attenuate ammonia water solution was used. It has two advantages. First, the appropriate pH was kept. Second, ammonia volatilized in drying process, which avoid the remainder of buffer solution. Spectrofluorometric measurements were used to characterize the nanoparticles. Free Rubpy dye exhibited an emission at 595 nm when excited at 435 nm in aqueous solution. However, the emission maximum of the nanoparticles shifted 4 nm to the longer wavelength compared with the pure Rubpy dye, indicating that the spectral characterization of the Rubpy dye did not change to a great extent when it was doped inside the nanoparticles. Photobleaching is known to be dependent on solvent interactions and is thought to occur as a bimolecular reaction between dissolved oxygen and the dye of the nanoparticles. The nanoparticles show significantly less photobleaching (7.8%) than the free dye (60%), even after continuous excitation for 60 min. This result, coming with the experimental phenomenon suggests that the silica shell surrounding the dye molecule acts as a barrier to protect dye from the surrounding environment (Hun and Zhang, 2007).

The surface of silica nanoparticles has a large number of Si—OH result in a negatively charged silica shell, which can form stable electrostatic interactions with $Ru(bpy)_3^{2+}$ (Qian et al., 2010; Eltohamy et al., 2011; He et al., 2003, 2005). The electrostatic interactions are significantly stronger than other electrostatic interactions formed in some dyes, like types of fluorescein,

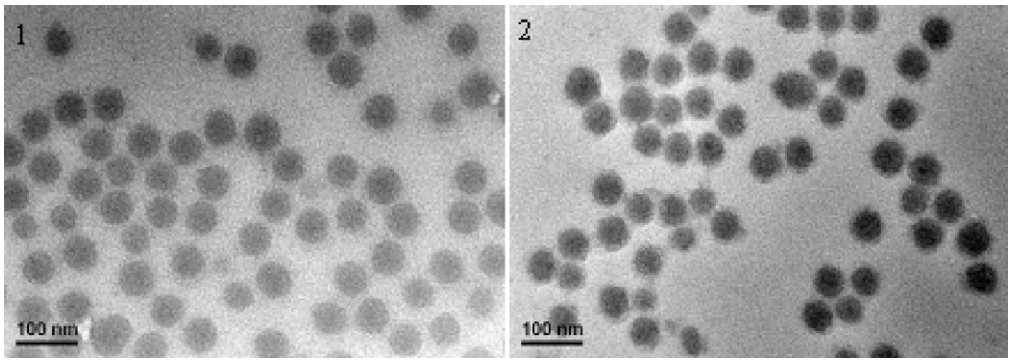

Fig. 2. TEM images of functionalized fluorescent core-shell nanoparticles at 30,000× magnification. (1) Free nanoparticles, (2) nanoparticles conjugated anti-EpCAM antibodies.

rhodamine. This leads to a lower leakage rate, higher fluorescence intensity, and better durability.

3.2. Covalent immobilization of anti-EpCAM monoclonal antibodies onto surface of the nanoparticles

The $NaIO_4$ method was a hardly seen method in the covalent crosslink between antibody and nanoparticles. In this method, the glycosyl of glycoprotein was oxidized to aldehyde group. Then the Schiff base was formed between aldehyde group and primary amino group on nanoparticles. After been deoxidized by $NaBH_4$, the Schiff base turned to —N=C— bond. The process of the $NaIO_4$ oxidation method is shown in Fig. 1. First, dihydroxy of glycosyl of monoclonal antibodies' Fc' segment was oxidized to aldehyde groups by $NaIO_4$ in 4 °C at pH 4.2, which Fab' segment of antibodies was hardly affected. It should be noted, however, that the $NaIO_4$-oxidized antibody can self-conjugate through their own amines if high-pH is used. Next, the oxidized antibodies were added in nanosuspension gradually in order to reduce the influence of antibodies' self-conjugation. After reacting for 30 min in 4 °C, the pH was adjusted to 7.6–7.8. Reacting for 20 h, Schiff base was formed between aldehyde groups of antibodies and amine groups of nanoparticles. If two amine groups were approached, two carbon=nitrogen double bonds could form which could be deoxidized to steady carbon—nitrogen single bonds. The antibodies should be in excess to form a monomolecular layer in nanoparticles, which reduces the non-specific adsorption of nanosensors. Because of the fixed reactant ratio and high extent of reaction, the labeling rate measured for this method was over 50%, a very high labeling rate. The other characteristic of this method was that crosslinker was not introduced, which eliminated the disadvantages of crosslinker for activities of antibodies.

In this study, two covalent immobilization methods were experimented to ascertain the influence of immobilization for antibodies capture ability. After the hydrolysis reaction of TEOS and APS, some amino groups have been introduced to the surface of nanoparticles. These amino groups made the modification and the bioconjugation of the nanoparticles easier. After mixing antibodies with nanoparticles for 30 min, the deposit appeared obviously. This phenomenon appeared in two methods. Compared with molecule biomarkers, decentralization of nanoparticles is very important for accomplishment of immobilization reaction. The deposit of nanosensors makes itself distinguish targets low performance. In the processing of immobilization reaction, electrostatic interaction is the important facts for decentralization of nanoparticles. Adjusting pH is the primary method to solve this problem. With an initial pH of 7.1, nanoparticles were dispersed thoroughly. After adding in antibodies, physical adsorption caused them to combine, which occurred before the covalent immobilization reaction. In this situation, the linkage between antibodies and nanoparticles introduced two effects into the reaction system. (1) The electric charge of the nanoparticles was changed. (2) Antibodies became the agent linking nanoparticles. These two factors made antibodies and nanoparticles deposit badly, so the pH of system was adjusted to 7.6–7.8 to facilitate dispersion. It is same in both methods.

In order to prove the difference of two methods, fluorescence microscopic imaging was used. Fig. 3 shows the fluorescence microscopic images of colo205 incubated with nanosensor using

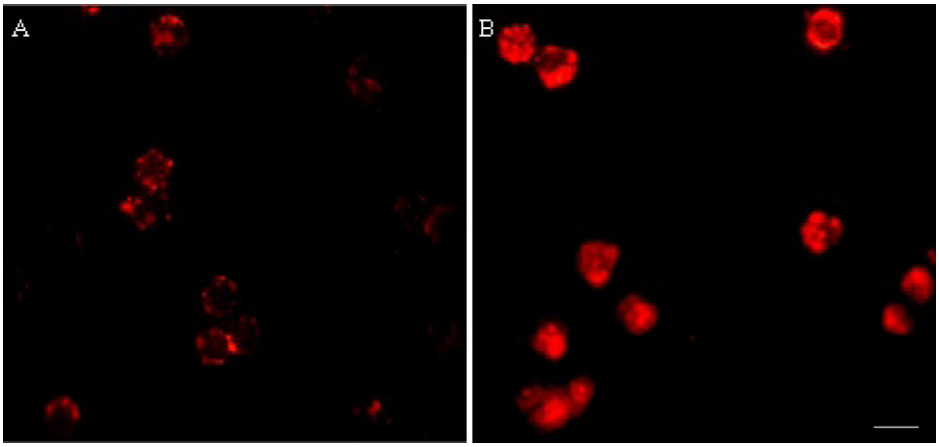

Fig. 3. Fluorescence microscopic images of colo205 with different nanosensors using different immobilization methods. Glutaraldehyde method (A), $NaIO_4$ oxidation method (B). Bar, 20 μm.

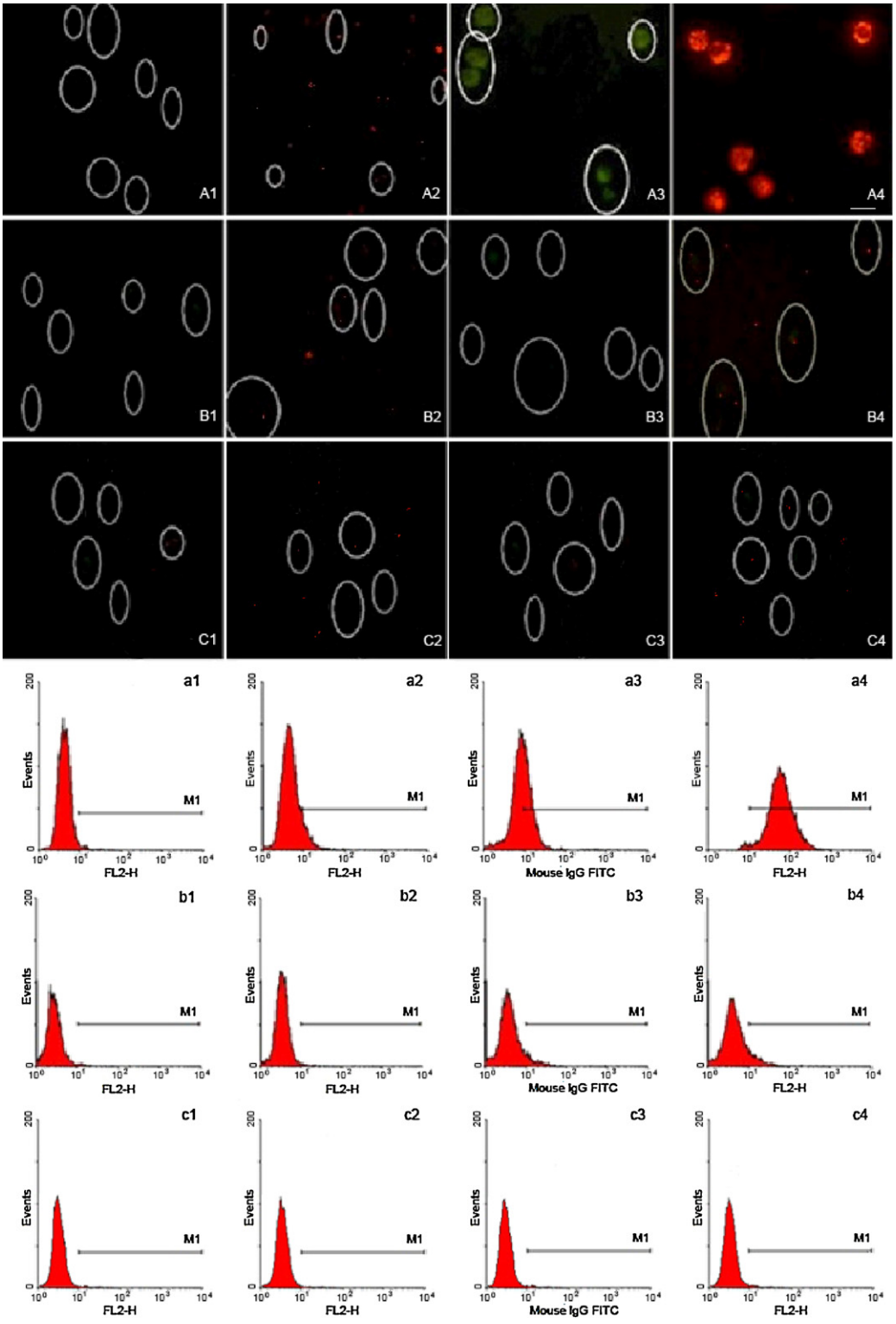

Fig. 4. The results of fluorescence microscopic images and flow cytometer. Colo205 (A1, a1), colo205 incubated with free nanoparticles (A2, a2), colo205 incubated with anti-EpCAM antibody marked iso thiocyanate fluorescein (A3, a3), colo205 incubated with anti-EpCAM antibody nanosensors using $NaIO_4$ oxidation method (A4, a4); sw480 (B1, b1), sw480 incubated with free nanoparticles (B2, b2) sw480 incubated with anti-EpCAM antibody marked iso thiocyanate fluorescein (B3, b3), sw480 incubated with anti-EpCAM antibody nanosensors using $NaIO_4$ oxidation method (B4, b4); NCM460 (C1, c1), NCM460 incubated with free nanoparticles (C2, c2), NCM460 incubated with anti-EpCAM antibody marked iso thiocyanate fluorescein (C3, c3), NCM460 incubated with anti-EpCAM antibody nanosensors using $NaIO_4$ oxidation method (C4, c4). Bar, 20 μm.

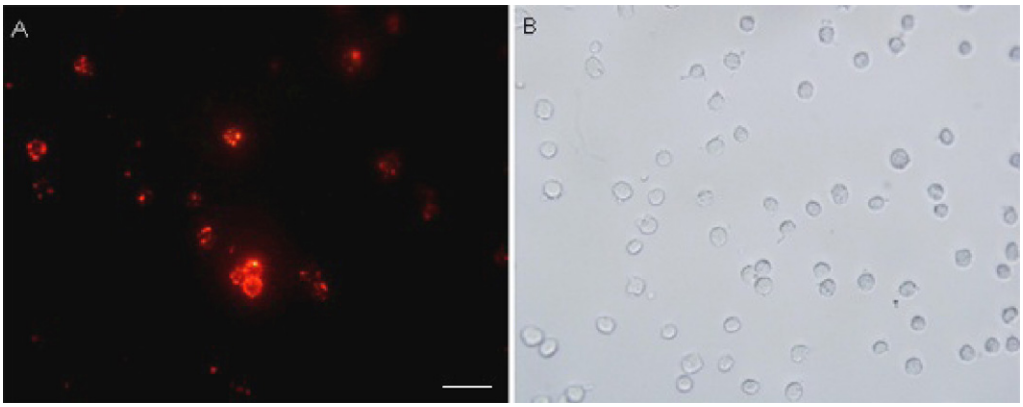

Fig. 5. Fluorescence microscopic image (A) and bright field image (B) mixing colo205 with sw480 incubated with anti-EpCAM antibody nanosensors. The excitation module of fluorescence microscope was WB mode. Bar, 50 μm.

glutaraldehyde method (A), and $NaIO_4$ oxidation method (B) respectively. The photos obviously show that the fluorescent signal of A was lower than B in detection of colo205. It proved that $NaIO_4$ oxidation method maintains the activities of antibodies better than glutaraldehyde method.

3.3. Detection of colon cancer cells

The fluorescence microscopic images of model cells are shown in Fig. 4. It was found that there were no fluorescent signals in A1, B1 and C1, for which no sensors were added in. As shown in Fig. 4A4, the nanosensors using the $NaIO_4$ oxidation method captured colo205 cells after 40 min incubation. Control experiments (Fig. 4B4 and C4) showed that the nanosensors were incubated with sw480 cells (a kind of EpCAM-deficient human colon cells) and NCM460 (normal human colon cells) for 40 min under the same conditions, no capture occurred. The anti-EpCAM antibody conjugated to nanoparticles was $NaIO_4$ oxidation method in A4, B4 and C4. The antibody marked iso thiocyanate fluorescein as biosensor is shown in Fig. 4A3, in which it could be seen that very low positive fluorescent signal was detected. B3 and C3 were the control experiment of A3. In Fig. 4A2, B2 and C2, there was no fluorescence because free nanoparticles were used.

When incubated in the presence of nanoparticles, the cells grew at a normal rate. For the demand of fluorescence microscopy and FCM analysis, the condition of incubation was suspension incubation with anti-EpCAM antibody conjugated nanosensors in 40 min at 4 °C. The advantage is that the nanosensors can react with the EpCAM in cell membrane fully. Because of the influence of cells' autofluorescence, the response time of CCD must be selected in fluorescence microscopy imaging. In order to ensure the same experimental conditions, we detected positive cells firstly in automatic mode of CCD. In this mode, the CCD could balance the intensity of signal and noise for the best quality of imaging. Then, other experiments were detected in the selected conditions which could be fixed in CCD's configuration. It is easy to find that the fluorescent intensity of colo205 cells incubated with nanosensor modified by anti-EpCAM antibodies was higher than fluorescent intensity of control groups, which proved that the nanosensor could distinguish cancer cells sensitively and specifically. Meanwhile, we found that the free nanoparticles congregated easily in the process of incubation with cells. This phenomenon demonstrated that the modification in nanoparticles' surface could decrease the non-specific adsorption between nanoparticles.

In order to confirm with the fluorescence microscopic results, FCM was also used. The results are shown in Fig. 4a1–c4. It can be seen that the fluorescent intensity using anti-EpCAM antibody nanosensors was higher than control groups in homocellular model. The fluorescent intensity of a4 was about ten times as high as b4. Compared with c4, the peak of b4 showed a slight shift to the right.

Being different from qualitative detection of fluorescence microscopy, FCM was used for a relative quantitative measure. The results of FCM histograms had the same situations as fluorescence microscopy imaging generally. Meanwhile, it has the advantages which fluorescence microscopy imaging does not possess. We can know the results by observing photos in fluorescence microscopy imaging intuitionally. However, the disadvantages of fluorescence microscopy imaging, such as small sample volume and non-quantitative detection, could not make us be convinced of the panorama of results. We can see in Fig. 4 that the fluorescent intensity of a4 was about ten times as strong as others. This result proved that the nanosensors can increase the detection sensitivity obviously. Meanwhile, the peak width of a4 was wider than others obviously. The phenomenon may be explained as the following reasons: (1) the distribution of antigen expressed in colo205 cells' surface was wider than sw480 cells and NCM460 cells. (2) The signal amplification ability of nanosensors made the low expression cells more recognizable. In Fig. 4b4, we also found out that the peak shift to right by a very small distance. Actually, EpCAM has very low expression rate in sw480 cells, which was not detected using anti-EpCAM antibody labeled iso thiocyanate fluorescein, also was not detected by fluorescence microscopy imaging using nanosensors. It was suggested that choice of equipment is also an important factor in detection of cancer cells. As other control experiments, NCM460 cells were also used for proving the reliability of the nanosensors (Fig. 4c1–c4). It can be seen that the histograms expressed negative results well, for the reason that NCM460 cannot express EpCAM antigen.

From the discussion of Figs. 3 and 4, it can be concluded that the detection capability of biosensors could be determined by two factors. One is the ability of capturing targets; the other is intensity of tracer signal detected, which includes choice of equipments.

3.4. Detection of system mixed colo205 and sw480

For future study, small amounts of colo205 were mixed with large numbers of sw480 as simulation of simple application. The detection of colo205 cells in system mixed with sw480 cells is shown in Fig. 5. The mixture ratio of two cells was 1:5 (colo205:sw480). Since sw480 was adherent cell, the shape of sw480 cell was not spherical after cell dissociation. The results of Fig. 5 demonstrated that the nanosensors could identify the positive colo205 cells well in this relatively complex system. This simple

experiment proved that colo205 cells were selected by anti-EpCAM antibody nanosensors in this environment, and made a good foundation for subsequent research.

4. Conclusion

A nanosensor for detecting colon cancer cells was established. Anti-EpCAM antibodies were conjugated to Rubpy-encapsulated core–shell silica nanoparticles surface by using a $NaIO_4$ oxidation method. The immobilization method maintained the activity of antibody excellently. This advantage proves nanosensor has highest sensitivity for detecting colon cancer cells. Owing to high sensitivity and specification of nanosensor, it not only can achieve the image of colon cancer cells but also shows the distribution and abundance of EpCAM in cells membrane. It provides also a possibility to quantify special membrane proteins on different regions of cells surface.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2012.02.044.

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