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ORIGINAL PAPER

Magnetic Fluorescent Composite Nanoparticles for the Fluoroimmunoassays of Newcastle Disease Virus and Avian Virus Arthritis Virus

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Abstract A new detection format for multiplexed analysis based on the use of magnetic fluorescent composite nanoparticles was presented in this paper. Two different antigens, Newcastle disease virus (NDV) antigen and Avian virus arthritis virus (AVAV) antigen, were conjugated to two kinds of magnetic fluorescent composite nanoparticles of different luminescent colors, while red-emitting CdTe QDs were attached to the antibody of NDV and AVAV. Both CdTe QDs-labeled antibodies and magnetic fluorescent composite nanoparticles labeled antigens were used to form a typical immunoreaction system for the detection of NDV and AVAV. Also a typical mixed separation format was realized, which showed the outstanding magnetic properties of composite nanoparticles and the broad application in immunoseparation.

Keywords Quantum dots · Fluoroimmunoassay · Magnetic fluorescent composite nanoparticles · Newcastle disease virus · Avian virus arthritis virus

Introduction

Newcastle disease (ND) and Avian virus arthritis (AVA), are caused by ND virus (NDV) and AVA virus (AVAV) respectively. Both of them are the most serious illnesses of birds, particularly chickens, for their high morbidity and high mortality in chickens of any age group. Both of ND and AVA are highly contagious diseases, it is vitally important to develop rapid and specific detection method for them. Because NDV and AVAV are both chicken contagious virus, so they are likely to coexist in the contaminated drinking water, feedstuff and tackles when the contagious diseases occur. So there are some practical needs to detect and separate these antigens in a single test.

Fluoroimmunoassays techniques using conventional fluorescent probes are essential to satisfy the growing demands of many biomedical fields. However, when conventional organic dyes are used for multiple analyses, their narrow excitation spectra make simultaneous excitation difficult, and their broad emission spectra with long tails at higher wavelengths introduce spectral cross-talk between different detection channels, making quantification difficult among different probes [1]. QDs as a new class of fluorescent probes instead of organic dyes have been widely used for real-time bioimaging because of their size-tunable photolumine-scence, resistance to photo-bleaching and broad absorption and sharp emission spectrum [2]. The application of water-soluble QDs in conjugating with biomolecules has been studied in the past several years [3–5].

Magnetic nanoparticles consisting of maghemite (r-F_2O_3) or magnetite (Fe_3O_4) have been of great interest not only for the study of fundamental magnetic properties, but also for biomedical application including bioseparation, biodetection, target therapy and immunoassays [6, 7]. Magnetic nanoparticles comprise of very eye-catching nanotechnology; it can conjugate with antibody, antigen or immunoglobulin, due to its good superparamagnetic and surface characteristics.

Because magnetic nanoparticles have excellent magnetic properties and QDs have excellent optical properties, so the

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composite nanoparticles embedded with QDs and magnetic nanoparticles will have wider application in biomedicine and biology including magnetic separation and detection of cancer cells, bacteria and viruses. The combination of magnetic and fluorescent properties is a new powerful tool, allowing manipulation by magnetic fields and visualization/detection by fluorescence, which could act as multi-targeting, multi-functional and multi-treating tools. It is obviously that the combination of magnetic and fluorescent properties in one nanocomposite would open up great prospects both in nano- and bio- technology. In recent years, The magnetic fluorescent composite nanoparticles are intriguing and reported by some researchers, such as polystyrene magentic beads with entrapped organic dyes/quantum dots [8, 9] or a shell of QDs [10], iron oxide particles coated with dye-doped silica shells [11], and silica nanoparticles embedded with iron oxide and QDs [12–15] have recently been reported. In most of the cases, the synthesis of particles with magnetic and fluorescent properties is complicated and expensive. An additional drawback is that their application is limited mostly to cellular separation, imaging drug delivery, and therapy. To our knowledge, there are very few papers have reported the use of magnetic fluorescent composite nanoparticles for multiplexed immunoassays so far.

In this paper, we present a new detection format for multiplexed analysis based on the used of magnetic fluorescent composite nanoparticles. The CdTe QDs were conjugated to NDV antibody and AVAV antibody by covalent coupling. The antibody-specific captured antigens were immobilized on the surface of different color magnetic fluorescent composite nanoparticles. Both CdTe QDs-labeled antibodies and magnetic fluorescent composite nanoparticles labeled antigens are used to form a typical immunoreaction system for the detection of NDV and AVAV. Also a typical mixed separation format was realized, which showed the outstanding magnetic properties of composite nanoparticles and the broad application in immunoseparation.

Experimental

Instrument

Fluorescence experiments were performed on a RF-5301 PC spectrofluorophotometer (Shimadzu Co., Japan), and a 1 cm path-length quartz cuvette was used to measure the fluorescence spectrum. A bath ultrasonic cleaner (Autoscience AS 3120, Tianjin, China) was used to disperse the composite nanoparticles. All pH measurements were completed using a PHS-3C pH meter (Hangzhou, China), and all optical measurements were carried out at room temperature under ambient conditions.

Reagents and chemicals

All chemicals used were of analytical reagent grade without further purification. N-hexane, tritonX-100, acetone, ethanol were purchased from Tianjin No. 1 Chemical Reagent Factory, China. Mercaptosuccinic acid (MSA) (99 %) was purchased from J&K Chemical Co. Tetraethoxysilane (TEOS), 3-aminopropyltrimethoxysilane (APS), and 3-(trihydroxysilyl)-propylmethylphosphonate (THPMP) were purchased from Sigma-Aldrich Chemical Co. Newcastle disease antigen virus (NDV antigen for short) (2 mg) and NDV antibody (2 mg), Avian virus arthritis virus (AVAV) antigen and AVAV antibody, were all obtained from Jilin Entry-Exit inspection and Quarantine Bureau. Poly (dimethyldiallylemmonium chloride) (PDDA, Mw=82,000 g/mol) was obtained from Sigma-Aldrich and used as received. The sulfo-NHS (*N*-hydroxysulfo-succinimide) and EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) was obtained from JK Chemical Co. Bovine serum albumin (BSA, 5 g) was obtained from Genview. All antigen powders were dissolved by 2 mmol/L phosphate-buffered saline solutions (PBS, pH 7.4) and stored at 0–4°C. All antigens, antibodies and other reagents used in the fluoroimmunoassay were diluted with PBS to the concentrations used only immediately prior to use. The water used in all experiments had a resistivity higher than 18 MΩ·cm.

Synthesis of magnetic fluorescent composite nanoparticles

Water compatible mercaptosuccinic acids capped CdTe QDs and water compatible monodisperse iron oxide nanoparticles used in this paper were synthesized as described in detail in previous paper [16]. Stable water compatible 3-mercaptosuccinic-capped CdTe QDs (0.02 mmol/L) with emission maximum at about 525 nm, 585 nm and 637 nm were used in the present experiments. Stable water compatible superparamagnetic iron oxide nanoparticles with the size of 5~12 nm were used in the present experiments [17]. The hysteresis loops of iron oxide nanoparticles were measured at temperatures of 300 K and the maximum saturation magnetization of iron oxide nanoparticles was bout 55 emu/g.

The magnetic fluorescent composite nanoparticles were synthesized by reverse microemulsion method at room temperature, which has been described in detail in ref.18. In this method, cyclohexane was used as a continuous phase, and Triton X-100 and *n*-hexanol were regarded as surfactant and co-surfactant respectively. Briefly, we mixed 15 mL cyclohexane, 3.5 mL Triton X-100, 3.5 mL *n*-hexanol, 800 μL aqueous solution of as-prepared CdTe QDs $_{585nm}$ and 200 μL aqueous solution of Fe_3O_4, 60 μL PDDA solution (0.075% v/v) and then 60 μL NH_4OH was added to form microemulsion. Then added 100 μL TEOS to

the microemulsion system to initiate the hydrolysis, the reaction progressed in the dark for 24 h of stirring. Then 10 μL APS and 40 μL THPMP were injected into the reaction system. The reaction system was then kept under stirring for one more day. The microemulsion was broken by adding 20 mL of acetone to the reaction system and the resultant precipitate was bifunctional composite nanoparticles which were washed in sequence with iso-propyl alcohol, ethanol and water. During each washing procedure, the silica composite nanoparticles dispersion was first subjected to high velocity centrifugation (8000 rpm, 5 min), followed by decantation of the supernatant and redispersion of the precipitate in the next solvent with the aid of supersonication. Ultimately, aqueous dispersions of the QDs $_{585nm}$-Fe_3O_4/SiO_2 composite nanoparticles (CN-1) were obtained for further experiments.

QDs $_{525nm}$-Fe_3O_4/SiO_2 composite nanoparticles (CN-2) were obtained by the same method as mentioned above. QDs $_{525nm}$ /SiO_2 composite nanoparticles (CN-3) were obtained by the same method as mentioned above, just without adding Fe_3O_4 nanoparticles in the process. The characters (the size, optical properties, magnetic properties) of the composite nanoparticles had been studied in detail in our previous work [18].

Covalent binding of the antigen with the composite nanoparticles

The antigen was connected with the amino groups on the surface of the composite nanoparticles by glutaraldehyde [19]. Amino-activated composite nanoparticles were suspended in phosphate buffer (pH 7.4). Taking 100 μL composite nanoparticles solution, 100 μL of 1% glutaraldehyde was added to the solution and vibrated for 30 min. Then certain amount of antigen was added to incubate with composite nanoparticles for 1 h at room temperature. After that, 10 μL of BSA (0.033 g/ml) was added to avoid non-specific binding, and the mixture was incubated at room temperature for 30 min. Then the mixture were centrifuged and washed with the PBS several times to remove the excess antigen and unbound glutaraldehyde. Finally, the composite nanoparticles labeled antigen was resuspended in 1 mL PBS. The CN-1 labeled NDV antigen, CN-2 labeled AVAV antigen and CN-3 labeled AVAV antigen were obtained in this way, respectively.

Covalent coupling of antibody and QDs_{637nm}

Sulfo-NHS and EDC were usually used to covalently attach carboxylic acid groups on QDs with amine containing antigens or antibodies. In this study, EDC and Sulfo-NHS with equal molar ratio were dissolved by 100 μL of PBS buffer (pH=7.4) under mild stirring. Then 100 μL of QDs_{637nm} was added. Here the carboxyl groups on QDs were first activated with EDC and modified with amine reactive sulfo-NHS. The same amount of the activated QDs_{637nm} were then mixed with various amount of antibody (1 mg/mL) from 1 μL to 120 μL, followed by incubation for 4 h in a shaker at room-temperature and then centrifuged at 12,000 rpm for 10 min. The CdTe QDs_{637nm} labeled antibody were then washed with PBS buffer three times by centrifugation/redispersion. Then the CdTe QDs_{637nm} labeled antibody was resuspended in 1 mL PBS. The QDs_{637nm} labeled NDV antibody and QDs_{637nm} labeled AVAV antibody conjugates were obtained in this way, respectively.

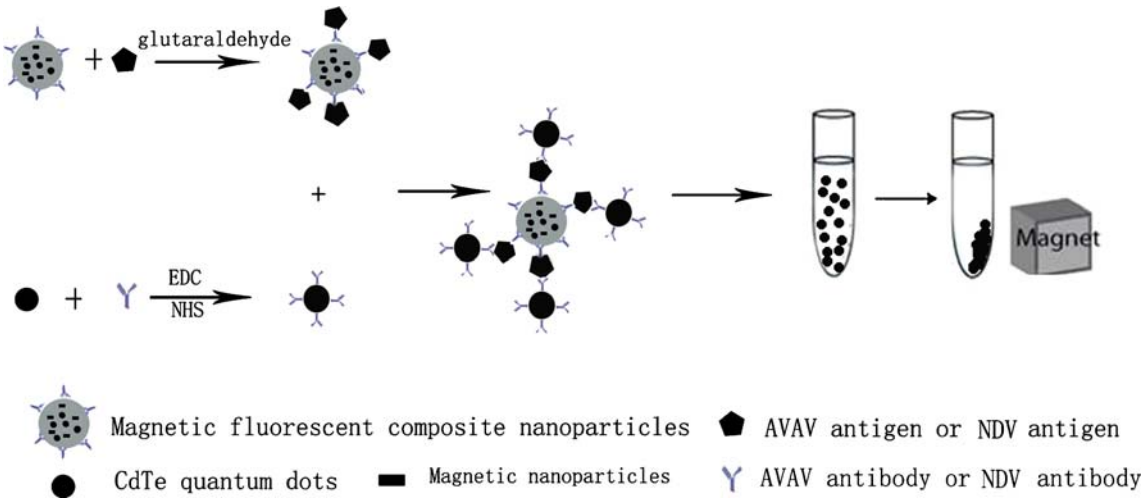
Immunoreaction on the surface of composite nanoparticles

Immunoreaction of composite nanoparticles labeled antigen and the corresponding QDs labeled antibody was carried by mixing the composite nanoparticles labeled antigen and the corresponding QDs labeled antibody in PBS buffer solution at room temperature, and the whole process was shown in the Schematic diagram 1. From Schematic diagram 1, it can be seen that the composite nanoparticles labeled antigen conjugated with the QDs labeled antibody through the antigen-antibody specific binding to form the immunocomplex on the surface of composite nanoparticles. The immunoreaction was incubated in a shaker for 12 h, and then the immunocomplex was separated by magnet and washed with PBS buffer for three times. The excess QDs labeled antibodies which were dispersed in the supernatant were discarded and then diluted the immunocomplex with PBS buffer. The QDs $_{585nm}$-Fe_3O_4/SiO_2 composite nanoparticles (CN-1) / NDV antigen/ QDs_{637nm} labeled NDV antibody immunocomplexes, QDs $_{525nm}$-Fe_3O_4/SiO_2 composite nanoparticles (CN-2) /AVAV antigen/ QDs_{637nm} labeled AVAV antibody immunocomplexes and QDs $_{525nm}$/SiO_2 composite nanoparticles (CN-3) /AVAV antigen/ QDs_{637nm} labeled AVAV antibody immunocomplexes were obtained using the same procedure.

Results and discussion

Fluorescence spectra of CdTe QDs and magnetic fluorescent composite nanoparticles

Spectra overlap is a limiting factor in target detection. For the purpose of minimizing spectral interference, we use green $QDs_{525\ nm}$ and orange QDs_{585nm} for composite nanoparticles doping and red QDs_{637nm} for antibody labeling. Figure 1 shows the emission spectra obtained from pure CdTe QDs and composite nanoparticles dispersed in PBS buffer solution. It can be seen that there are three distinct maximum emission wavelengths of QDs



Schematic diagram 1 Schematic diagram of the whole process of the analysis method based on the magnetic fluorescent composite nanoparticles

sharing a broad overlapping excitation spectrum when excited at 440 nm simultaneously. Compared with the free QDs ($QDs_{525\ nm}$ and QDs_{585nm}), we can see that after silica coating, the maximum emission peak of QDs became blue shift (at 515 nm and 573 nm, respectively) and broader obviously. It had been demonstrated that the blue shift was due to the corrosion of QDs during silica deposition. Since thiol ligands must be completely removed from their surfaces, leaving the QDs unprotected, thus resulting in this blue-shift in the maximum of emission spectra [20].

Formation of QDs_{637nm}/antibody conjugates

In order to studies the optimality conjugation amount of antibody to QDs, 1~120 μL NDV antibody (1 mg/ml) was added to 100 μL activated QDs_{637nm}, respectively. And followed by incubation for 4 h and then centrifuged at 12,000 rpm for 10 min to discard the excess QDs exist in this solution. Figure 2 shows the fluorescence intensity of QDs_{637nm}/ NDV antibody increases obviously with increasing concentrations of NDV antibody, which is due to the amount of QDs conjugated with antibody will increased with the increase of the amount of antibody. When the volume of NDV antibody exceeds 100 μl, the fluorescence intensity of QDs_{637nm}/ NDV antibody do not change anymore, which indicates that 100 μL of activated QDs are completely conjugated with the antibodies, so the fluorescent intensity was almost the same when 100 μL and 120 μL of antibody was added. In this study, 100μL of NDV antibody was chosen, and QDs_{637nm} (2×10^{-6}mmol/mL)/ NDV (0.1 mg/mL) conjugates can be obtained.

For AVAV antibody, the same amount of antibody as NDV antibody was used in the study. Figure 3 shows the emission spectra obtained from pure $QDs_{637\ nm}$ and $QDs_{637\ nm}$-antibody ($QDs_{637\ nm}$-NDV antibody and $QDs_{637\ nm}$-AVAV

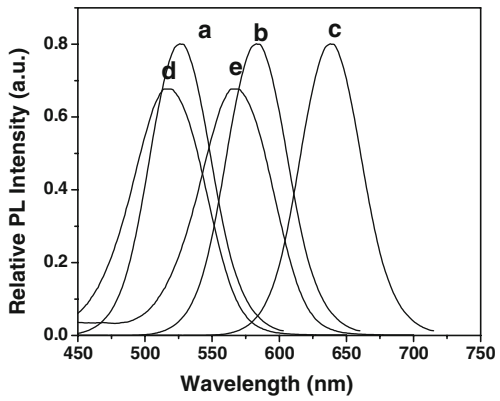


Fig. 1 Fluorescent emission spectra of mercaptosuccinic acid capped CdTe nanocrystals (a, b, c) and magnetic fluorescent composite nanoparticles (d, e) in PBS aqueous solution with the emission wavelength of 525 nm (a), 585 nm (b), 637 nm (c), 515 nm (d) and 573 nm (e)

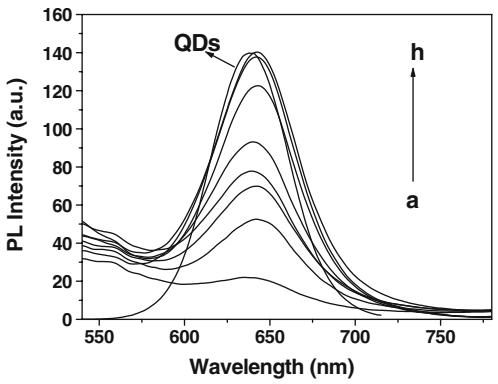


Fig. 2 The fluorescence emission spectra of QDs_{637nm}-NDV antibody with a series of different volume of NDV antigen added: (a) 1 μL; (b) 5 μL; (c) 10 μL; (d) 20 μL; (e) 50 μL; (f) 80 μL; (g) 100 μL; (h) 120 μL

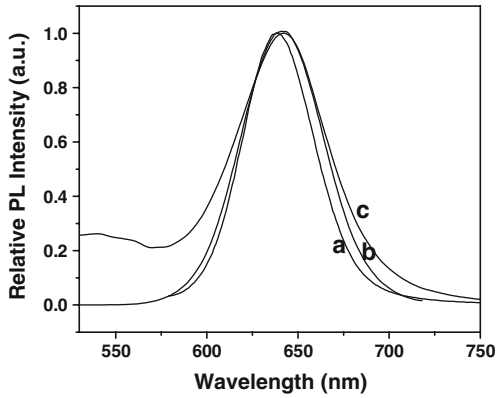


Fig. 3 Fluorescence emission spectra of QDs_{637nm} (**a**), QDs_{637nm} -NDV antibody (**b**), QDs_{637nm} -AVAV antibody (**c**)

antibody) bioconjugates dispersed in PBS buffer solution at pH 7.4. From Fig. 3, it can be seen that the emission peak of the pure QDs is at 637 nm, the emission peak of QDs–antiboday bioconjugates undergoes a red shift (642 nm), indicating the intrinsic spectral width becomes wider. The changes of the fluorescences spectra result from the shortened distances between QDs owing to the covalent coupling between QDs and antiboday, which increase dipole–dipole interaction between QDs and hence cause a larger Stokes-loss [20, 21].

Effect of reaction time

In this study, the effect of reaction time on the fluorescence emission spectra of the QDs labeled NDV antibody/ NDV antigen/ composite nanoparticles immunoreaction system was investigated at room temperature. For this

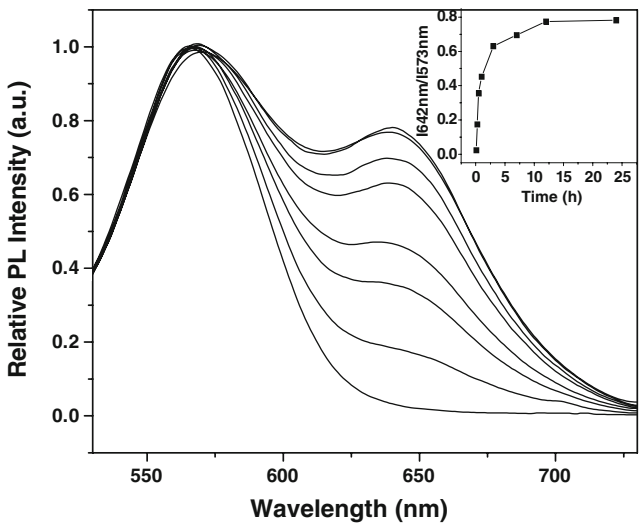


Fig. 4 The effect of reaction time on the fluorescence emission spectra of magnetic fluorescent composite nanoparticles /NDV antigen/NDV antibody immuoreaction system: (A)5 mins; (B) 15 mins; (C) 30 mins; (D) 1 h; (E) 3 h; (F)7 h.; (G) 12 h; (H) 24 h

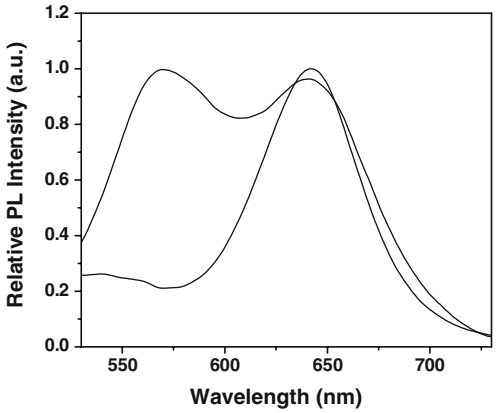


Fig. 5 Fluorescence emission spectra of NDV antigen/ magnetic fluorescent composite nanoparticles after immunoreaction with NDV antibody (**a**), and AVAV antibody (**b**)

purpose, the reaction time of $QDs_{637\ nm}$ labeled NDV and NDV antigen immobilized composite nanoparticles (CN-1) was changed from 5 min to 24 h. The results are shown in Fig. 4, the inset in Fig. 4 shows a graph of the reaction time versus fluorescence intensity at 642 nm. From Fig. 4, it can be seen that the fluorescence intensity at 642 nm increases rapidly with the reaction time during the first 12 h and showed no changes after the time exceeded 12 h. In this study, reaction time of 12 h was adopted for further study.

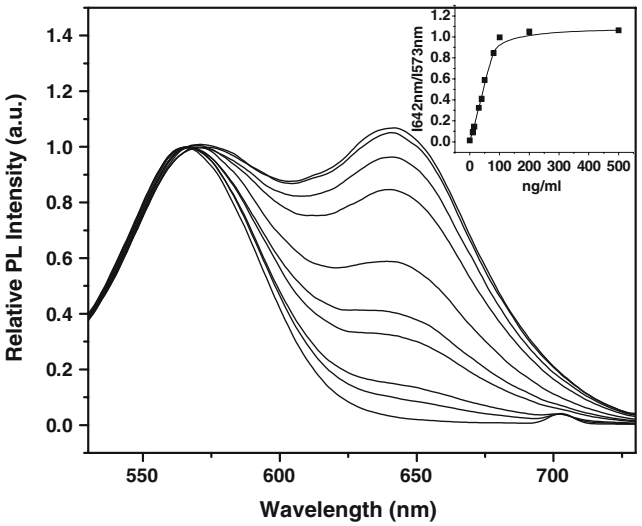


Fig. 6 The fluorescence emission spectra of QDs-NDV antibody/ NDV antigen/ magnetic fluorescent composite nanoparticles (573 nm) immunoreaction system with a series of different concentration of NDV antigen added: (A) 0 ng/mL; (B) 10 ng/mL; (C) 15 ng/mL; (D) 30 ng/mL; (E) 40 ng/mL; (F) 50 ng/mL; (G) 80 ng/mL; (H) 100 ng/mL; (I) 200 ng/mL; (J) 500 ng/mL. The inset shows the curve relationship between fluorescent intensity at 642 nm of composite nanoparticles after immunoreaction and the concentration of NDV antigen

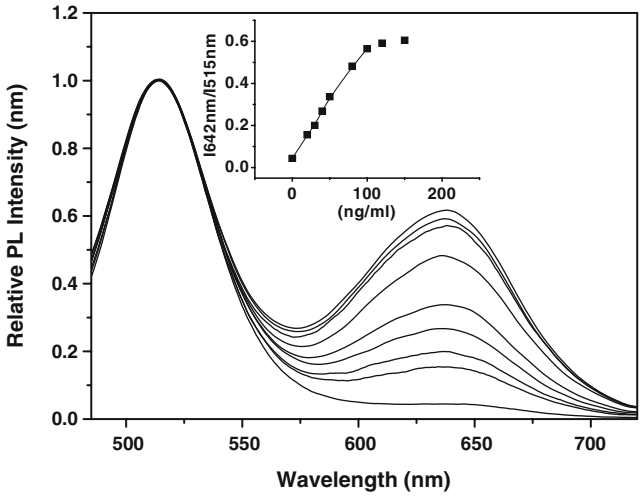


Fig. 7 The fluorescence emission spectra of QDs-AVAV antibody/ AVAV antigen/ magnetic fluorescent composite nanoparticles (515 nm) immunoreaction system with a series of different concentration of AVAV antigen added: (A) 0 ng/ mL; (B)20 ng/ mL; (C) 30 ng/ mL; (D) 40 ng/ mL; (E)50 ng/ mL; (F) 80 ng/ mL; (G)100 ng/ mL; (H) 120 ng/ mL; (I) 150 ng/mL. The inset shows the curve relationship between fluorescent intensity at 642 nm of encoded beads after immunoreaction and the concentration of AVAV antigen

Nonspecific binding of composite nanoparticles/antigen/antibody immunoreaction system

In order to study the nonspecific binding of composite nanoparticles/antigen /antibody immunoreaction system, $QDs_{637\ nm}$ labeled NDV antibody and composite nanoparticles (CN-1) / NDV antigen were mixed by using the procedure described above. $QDs_{637\ nm}$ labeled AVAV antibody and composite nanoparticles (CN-1)/NDV antigen were mixed according to the same procedure. Fluorescence spectra of the immunoreaction system are shown in Fig. 5. It can be seen that the luminescence peaks at 573 nm were observed in the system of QDs labeled NDV antibody/NDV antigen /composite nanoparticles (CN-1), but the luminescence peak at 573 nm does not appear in the system of QDs labeled AVAV antibody/NDV antigen composite microshperes (CN-1). This indicates that there is no immunoreaction between AVAV antibody and NDV antigen. The nonspecific binding is probably negligible in this study. It also confirmed the interaction between NDV antibody and NDV antigen on the composite nanoparticles surface.

Immunoassay for the detection of NDV antigen and AVAV antigen

In order to detect NDV antigen and AVAV antigen, various amounts of NDV antigen and AVAV antigen were used to conjugate with 100 μL of CN-1 composite nanoparticles and CN-2 composite nanoparticles respectively. After conjugation, the antigen-conjugated magnetic fluorescent composite nanoparticles (CN-1/NDV antigens and CN-2/AVAV antigen) were separated by using a magnet, and followed by addition of 200 μL corresponding $QDs_{637\ nm}$ labeled antibody. After immunoreaction, the immunocomplex was separated by magnet to discard the excess QD-antibody, and then measured the fluorescence spectra of the QD-antibody-antigen-composite nanoparticles immunocomplex.

Figure 6 shows the fluorescent spectra of f CN-1 /NDV antigen/QDs labeled NDV antibody immunocomplexed with different concentration of NDV antigens deposited on the composite nanoparticles. If the immunoreactions between antigens immobilized on the functional composite nanoparticles and antibodis occurred, the QD-labeled antibodies can be captured by antigen which was on the surface of biofunctional composite nanoparticles. From Fig. 6, it can be seen that the red emitting QDs_{637nm} peak of QDs labeled NDV antibody appeared in the fluorescence spectra of CN-1/NDV antigen/QDs-NDV antibody system obviously. For the system of CN-1/NDV antigen/QDs-NDV antibody, it can be seen that the fluorescence intensity at 642 nm increases linearly with the increasing of the

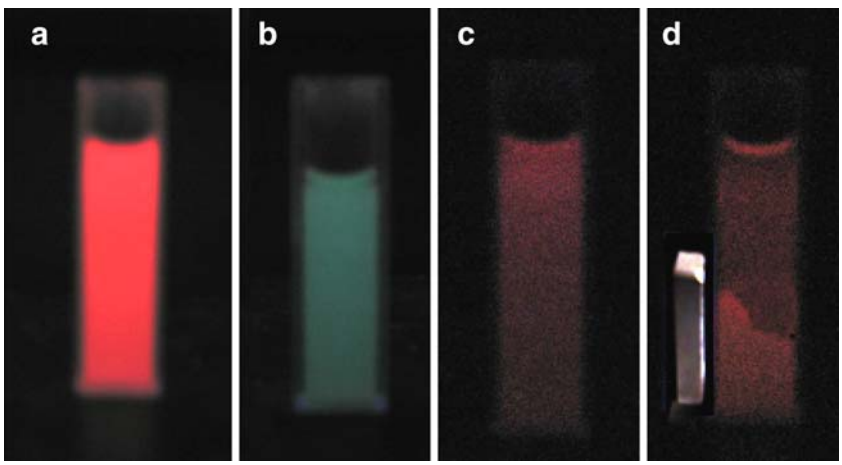


Fig. 8 The photographs of magnetic fluorescent composite nanoparticles under UV light: (a) CN-1/NDV antigen/QDs-NDV; (b) CN-3 /AVAV/ QDs-AVAV; (c) The mixture of CN-1/ NDV antigen/QDs-NDV and CN-3 /AVAV/ QDs-AVAV; (d) The mixture of CN-1/NDV antigen/QDs-NDV and CN-3 / AVAV/ QDs-AVAV with an external magnetic field

concentration of NDV antigen in the range of 0–500 ng/mL (Fig. 6). When the concentration of NDV antigen reaches 200 ng/mL, the fluorescence at 642 nm reaches a plateau. The insert in Fig. 6 shows the curve relation between concentration of NDV antigen and fluorescent intensity at 642 nm. The linear regression equation is as follows: $I_{642\ nm}/I_{573\ nm} = 0.0131 + 7.81 \times 10^{-3}\ C_{NDVantigen}$(ng/mL), and the coefficient of correlation is 0.996, the limit of detection for immunoassay is 1.5 ng/mL NDV antigen.

For the system of CN-2 /AVAV antigen/QDs-AVAV antibody, the same process was used as the system of CN-1/NDV antigen/QDs-NDV antibody. From Fig. 7, it can be seen that the fluorescence intensity at 642 nm also increases gradually with the increasing of the concentration of AVAV antigen in the range of 0–150 ng/mL. The fluorescence intensity at 642 nm reaches a plateau when the concentration of AVAV antigen reaches 120 ng/mL. Insert in Fig. 7 shows the curve relation between concentration of AVAV antigen and the fluorescent intensity at 642 nm. The linear regression equation is as follows: $I_{642\ nm}/I_{515\ nm} = 0.0434 + 5.23 \times 10^{-3}\ C_{AVAV\ antigen}$(ng/mL), and the coefficient of correlation is 0.982, and the limit of detection for immunoassay is 2.1 ng/mL AVAV antigen.

In comparison, the reported detection limits of established techniques for determination of these virus antigens is 10 ng NDV antigens for conventional PCR [22]; and 8 ng AVAV antigens for Dot-ELISA [23]. With respect to provide a bioassay platform for high sensitivity detection and quantification of trace virus, the composite nanoparticles offer a higher sensitivity (1.5 ng/ml for NDV antigens and 2.1 ng/ml for AVAV antigens), which will provide a new effectively method for detection of virus and disease diagnosis.

To illustrate the outstanding magnetic properties of magnetic fluorescent composite nanoparticles, a typical mixed separation format was realized through the separation of CN-1/NDV antigen/ $QDs_{637\ nm}$-NDV antibody from the mixture of CN-1/NDV antigen/ $QDs_{637\ nm}$ -NDV antibody and CN-3 /AVAV/ $QDs_{637\ nm}$ -AVAV antibody. In this mixture, the CN-1 was magnetic fluorescent composite nanoparticles, while CN-3 was only fluorescent composite nanoparticles. From Fig. 8, it can be seen that the system of CN-1/NDV antigen/$QDs_{637\ nm}$-NDV antibody and CN-3 /AVAV/ $QDs_{637\ nm}$ -AVAV antibody disperse homogeneously in aqueous solution in the absence of an external field (Fig. 8a and b), and the mixture (Fig. 8c) showed the mixed color of CN-1, CN-3 and $QDs_{637\ nm}$. When the external magnetic field was applied, the CN-1/NDV antigen/ $QDs_{637\ nm}$-NDV antibody immunocomplexes rapidly responded to the external magnetic field and get crowded near the point where the magnetic field existed actually (Fig. 8d). When the magnetic field was removed, alignments of the CN-1/NDV antigen/ $QDs_{637\ nm}$-NDV antibody were immediately broken and they dispersed again within few seconds by virtue of ultrasonic, which showed the outstanding magnetic properties of composite nanoparticles and the broad application prospects in immunoseparation.

Conclusions

In the current study, we have reported a novel strategy for fluoroimmunoassay with magnetic fluorescent composite nanoparticles, which were prepared by reverse microemulsion methods. The magnetic fluorescent composite nanoparticles, which contain the CdTe and Fe_3O_4 nanoparticles, are a new powerful tool in biological application. In this study, the magnetic fluorescent composite nanoparticles were conjugated with antigen by covalent coupling, and the corresponding antibodies were labeled QDs via covalent coupling. Then the QDs-NDV antibody/NDV antigen/ composite nanoparticles (CN-1) and QDs-AVAV antibody/AVAV antigen/ composite nanoparticles (CN-2) immunocomplexes were formed. The magnetic fluorescent composite nanoparticles were used to detect two kinds of antigens effectively in fluoroimmunoassays in this study. In comparison with QDs based immunoassays, the use of magnetic fluorescent composite nanoparticles provide a new and better approach while possessing the abilities of fluorescent marker and immune separation. It would help epidemiologists to trace the origin and epidemics of the ND virus and AVA virus. Further study will be extended to multiplexed optical labeling and enrichment analysis of proteins, medical diagnosis and analysis of genes in analytical biochemistry. In addition, magnetic fluorescent composite nanoparticles will have application in the development of fluoroimmunoassays and immunoseparation devices.

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References

1. Kuo YC, Wang Q, Ruengruglikit C, Yu H, Hua QR (2008) Antibody-conjugated CdTe quantum dots for escherichia coli detection. J Phys Chem C 121:4818
2. Kim J, Park K, Hahn S (2008) Effect of hyaluronic acid molecular weight on the morphology of quantum dot-hyaluronic acid conjugates. J Bio Macromolecules 42:41
3. Wang S, Mamedova N, Kotov NA, Chen W (2002) Antigen/antibody immunocomplex from CdTe nanoparticle bioconjugates. Nano Lett 2:817

4. Lin ZB, Cui SX, Zhang H, Chen QD, Yang B, Su XG, Zhang JH, Jin QH (2003) Studies on quantum dots synthesized in aqueous solution for biological labeling via electrostatic interaction. Anal Biochem 319:239
5. Clapp AR, Goldman ER, Mattoussi H (2006) Capping of CdSe–ZnS quantum dots with DHLA and subsequent conjugation with proteins. Nat Protoc 1:1258
6. Zhao M, Josephson L, Tang Y, Weissleder R (2003) Magnetic sensors for protease assays. Angew Chem Int Ed Engl 42:1375
7. Doyle PS, Bibette J, Bancaud A, Viovy JL (2002) Self-assembled magnetic matrices for DNA separation chips. Science 295:2237
8. Mulvaney SP, Mattoussi HM, Whitman LJ (2004) Incorporating fluorescent dyes and quantum dots into magnetic microbeads for immunoassays. Biotechniques 36:602
9. Gaponik N, Radtchenko I, Sukhorukov G, Rogach A (2004) Luminescent polymer microcapsules addressable by a magnetic field. Langmuir 20:1449
10. Wang DS, He JB, Rosenzweig N, Rosenzweig Z (2004) Superparamagnetic Fe_2O_3 Beads-CdSe/ZnS quantum dots core-shell nanocomposite particles for cell separation. Nano Lett 4:409
11. Levy L, Sahoo Y, Kim KS, Bergey EJ, Prasad PN (2002) Nanochemistry: synthesis and characterization of multifunctional nanoclinics for biological applications. Chem Mater 14:3715
12. Yi DK, Selvan ST, Lee SS, Papaefthymiou GC, Kundaliya D, Ying JY (2005) Silica-coated nanocomposites of magnetic nanoparticles and quantum dots. J Am Chem Soc 127:4990
13. Salgueirino-Maceira V, Correa-Duarte MA, Spasova M, Liz-Marzan LM, Farle M (2006) Composite silica spheres with magnetic and luminescent functionalities. Adv Funct Mater 16:509
14. Santra S, Bagwe RP, Dutta D, Stanley JT, Walter G, Tan W, Moudgil BM, Mericle RA (2005) Synthesis and characterization of fluorescent, radio-opaque, and paramagnetic silica nanoparticles for multimodal bioimaging applications. Adv Mater 17:2165
15. Sathe TR, Agrawal A, Nie SM (2006) Mesoporous silica beads embedded with semiconductor quantum dots and iron oxide nanocrystals: Dual-function microcarriers for optical encoding and magnetic separation. Anal Chem 78:5627
16. Wang C, Ma Q, Su XG (2008) Synthesis of CdTe nanocrystals with ercaptosuccinic acid as stabilizer. J Nanosci & Nanotechnol 8:4408
17. Lu ZY, Wang G, Zhuang JQ, Yang WS (2006) Effects of the concentration of tetramethylammonium hydroxide peptizer on the synthesis of Fe_3O_4/SiO_2 core/shell nanoparticles. Colloids and Surfaces 278:140
18. Wang GN, Wang C, Dou WC, Ma Q, Yuan PF, Su XG (2009) The synthesis of magnetic and fluorescent bi-functional silica composite nanoparticles via reverse microemulsion method. J Fluoresc In press: doi:10.1007/s10895-009-0493-8
19. Ye Z, Tan M, Wang G, Yuan J (2004) Preparation, characterization, and time-resolved fluorometric application of silica-coated. Anal Chem 76:513
20. Guo J, Yang WL, Wang CC, He J, Chen JY (2006) Poly (*N*-isopropylacrylamide)-coated luminescent/Magnetic silica microspheres: preparation, characterization, and biomedical applications. Chem Mater 18:5554
21. Wuister SF, Swart I, Driel F, Hickey SG, Donegá CDM (2003) Highly luminescent water-soluble CdTe quantum dots. Nano Letter 3:503
22. Tan SW, Omar AR, Aini I, Yusoff K, Tan WS (2004) Detection of Newcastle disease virus using a SYBR green I real time polymerase chain reaction. Acta Virol 48:23
23. Shien JH, Yin HS, Lee LH (2000) An enzyme-linked immunosorbent assay for the detection of antibody to avian reovirus by using protein σ B as the coating antigen. Res Vet Sci 69:107