Phosphorylcholine-protected Nanoparticles Functionalized with Both Magnetic and Photoluminescent Properties

Y. C. Chung*, C. J. Chen**

*Department of Chemical and Material Engineering, National University of Kaohsiung, Kaohsiung, 811 Taiwan R.O.C., ycchung@nuk.edu.tw

**Institute of Biotechnology and Chemical Engineering, I-Shou University, Kaohsiung County, 840 Taiwan R.O.C., 8750018@pchome.com

ABSTRACT

With an aim to prepare biomedical nanoparticles with core-shell morphologies displaying both magnetic and photoluminescent properties, a zinc sulfide thin layer coated on an iron oxide nanoparticle surface was utilized and protected by a biocompatible surfactant, 11-mercaptoundecylphosporylcholine (SPC), to stably disperse in biology systems. TEM image confirmed the ferric oxide core and ZnS shell morphologies of nanoparticle with 9 nm of diameter. An emission ZnS band centered at 425 nm (excited at 330 nm) was found from the photoluminescence spectrum of the nanoparticles. The nanoparticles mixed with albumin in aqueous buffer solution were analyzed using HPLC separation and TEM observation, illustrating a weak interaction between proteins and nanoparticles. The culture of nanioparticles with 3T3 fibroblast cells based on various culturing periods and temperatures showed the particles internalized quickly by cells from observation by confocal microscope.

Keywords: nanoparticles, superparamagnetism, photoluminescent, biocompatible

1 INTRODUCTION

The synthesis of ultrasmall magnetic nanoparticles with uniform size distribution and specific function is getting essential because of its wide applications in biology and medicine, for example the contrast agents for magnetic resonance imaging (MRI), magnetic separations for oligonucleotides, proteins, cells, and other biocomponents, magnetically guided drug delivery systems and so on. For those applications on intracellular chemotherapy and disease detection, a grand challenge of nanoparticles was met to carry specific antibiotic, ligand or genes that can be recognized and internalized by cells. Some liposomes and nanoparticles have brought much attention for developing nano-sized carriers. In general, the biocompatibility of phospholipid coats on liposomes provides good affinity toward cells, however, the natural opsonization of liposomes and their osmotic fragility can destabilize them, leading to leakage of the entrapped drug. Meanwhile, large studies of nanoparticles have focused on the polymeric nanospheres, however, the release rate of drugs were highly correlated with the degradation of the polymers, satisfied with long term applications. On the other hand, the superparamagnetic nanoparticles display the ability to be induced and guided to the near zone of pathological changes in order to carry out the active target transport of nanoparticles. Unfortunately, general magnetic nanoparticles will be surrounded and adsorbed by proteins as soon as contacting with blood though anionic/cationic charges may be dispersed on the particle surfaces. These complexes will precipitate immediately or be attacked by endocytosis of immunosystem in a short time, leading to unavailable doses. Therefore, the biocompatibility is a key issue of the development of biomedical nanoparticles. Furthermore, with a desire to detect and study the nanoparticles related with cells in a convenient way, the nanoparticles with photoluminescence (i.e. quantum dots, QDs) were prepared to recognize the changed cells or tissues more rapidly. There have been many studies concentrated on the ultrasensitive nanobiosensor using CdS, CdSe, ZnS QDs recently. For combining the advantages of above-mentioned functions in one nanoparticle, it was intended to prepare the biomimicking monolayer-protective nanoparticles with superparamagnetic and photoluminescent properties for the purpose of targeted drug delivery.

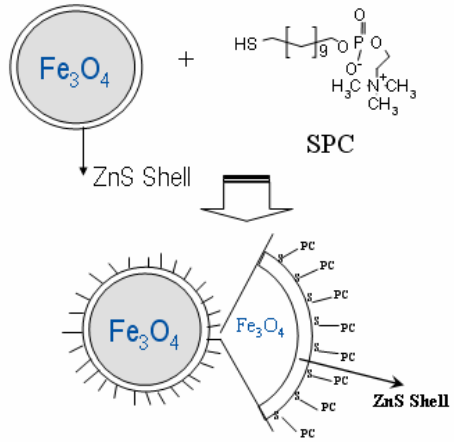

Fig. 1. Structure of the Fe3O4(core)/ZnS(shell) nanoparticle and PC Monolayer protection for the outmost surfaces.

2 EXPERIMENTAL

2.1 Synthesis of Core-shell Nanoparticles

The superparamagnetic magnetite was synthesized using a procedure as the literature reported [1]. Briefly, 2 M of FeCl3 solution was mixed with 1M of Sodium sulfite for about 15 min and then 80 ml of 1.8 % ammonia water were poured into the flask under sonication (20 % of 100 W, Branson) for 30 min. Deionized water and ethanol rinse twice as well as centrifugation operation separated particles from the solution. After magnetic precipitation and centrifugation of nanoparticles for separation, Fe_3O_4 (8 mg) pre-adsorbed with zinc acetate overnight was dispersed in 100 ml deionized water and then sonicated with ultrasonic horn at 80 °C for 3 h after addition of thioacetamide [2]. Afterward, the phospholipid derivative, 11-mercaptoundecylphosphorylcholine (SPC), synthesized by reduction of 1 mmol phosphorylcholine-ended disulfide (developed by our lab [3]) using sodium borohydride, was added into the suspension under mild shake overnight to stabilized the outmost ZnS surfaces of nanoparticles. Separation processes by centrifugation or magnet, washing repeatedly with distilled water and then drying in a vacuum gave a dark brownish powder.

2.2 Characterization

The nanoparticles were observed from TEM analysis for their morphologies and size distribution. Superconducting quantum interference device magnetometer (SQUID) was used to measure the magnetic property of nanoparticles. The surfaces of nanoparticles were characterized by X-ray photoelectron spectrometer (XPS) and the bulk properties were measured by UV and fluorescent spectrophotometers. X-Ray diffraction analysis was use to explore the magnetite crystallinity.

2.3 Protein Adsorption Test

The nanoparticle suspensions were incubated with 1 ml of Fetal Bovine Serum for 5 min at 37 °C in albumin-pretreated vials. After magnetic separation (6000 gauss) and rinse of the particles several times with distilled water, the adsorbed proteins were eluted from the particle surfaces by a protein solubilizing solution and then applied to the SDS-PAGE (polyacryamide gel) and high performance liquid chromatography (using C8 column)

In continuing with the above steps of treatments, the nanoparticle-protein complexes were fixed with 2.5% glutaldehyde, dehydrated by ethanol, dyed by OsO4. TEM was used to visualize the adsorption of proteins.

2.4 Cell Uptake Measurement

Fibroblast cells were used and grown in 35 mm culture dishes using nanoparticle-added DMEM medium for incubation at 4 °C for 1 hr. Other cases were under the same condition and then changed to 37 °C for 15 h to restore the endocytosis activity. The particle-cell samples were fixed onto glass slides being visualized by fluorescence microscope and confocal microscope.

3 RESULTS AND DISCUSSION

Basically, co-precipitation method in alkali solution can be employed to synthesize the nano-sized magnetite in a rapid stirring system. However, ultrasound effect arises from acoustic cavitation led to better dispersion for nanoparticles than using mechanical stir. Agglomeration and oxidation occurred during mechanical stirring process rendered particles to precipitate and no more disperse in aqueous solution. ZnS coating was also carried out by sonication under control of reaction temperature. Gedanken supposed that ultrasound induces some regions to promote reactions [2], especially in the interfacial zone and the bulk solution. Detailed discussion about the mechanism was mentioned that sonochemical implantation of Zn^{2+} into silica enhanced the zinc acetate coated on the particle surfaces. As our results showed, zinc acetate can be immobilized onto the magnetite surfaces firmly after rinsing, indicating the effect of ultrasound irradiation.

Transmission electron micrograph (TEM) and corresponding particle size distribution are shown in Figure 2. The morphology of the particles is roughly spherical and homogeneous. Some of particles are agglomerates while the average particle size is about 9 nm with around 1 nm thickness of ZnS shell.

The optical absorption spectrum of the sample shows in Figure 3-(1). The bulk semiconductor photo absorption corresponds at about 344 nm while the single absorption peak observed at 265 nm, suggesting a narrow size distribution of nanoparticles. Such a strong and narrow peak is known to arise due to quantum confinement effect.

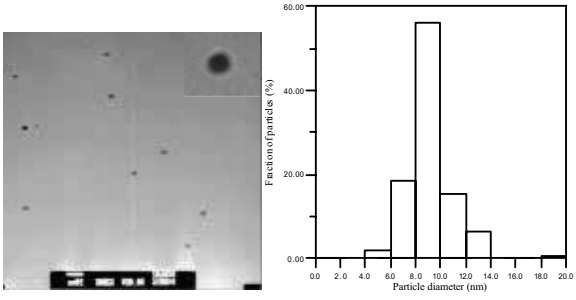

Fig. 2. TEM photograph for core/shell nanoparticles with around 9 nm in diameter and 1 nm thick in shell. The small window shows the high amplification view for the structure of nanoparticles and the right shows the size distribution from estimating more than 400 counts. Average diameter is 9.5 ± 2.1 nm.

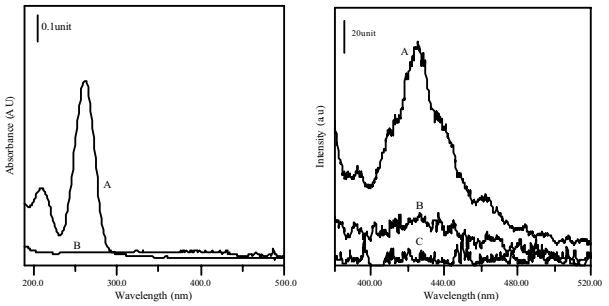

Fig. 3. (1) UV absorption for (A) Fe_3O_4(core)/ZnS(shell) with a sharp absorption peak at 265 nm, and (B) pure Fe3O4 without apparent absorption. (2) PL spectra at about 425 nm for (A) Fe_3O_4(core)/ZnS(shell), (B) pure Fe_3O_4 and (C) quartz only. (excited at 330 nm wavelength of Xe light)

Figure 3-(2) shows the photoluminescence (PL) spectra of the nanoparticles. An emission band centered at 425 nm can be seen and attributed to the ZnS thin layer rather than to the Fe3O4 and background. For most semiconductor nanocrystals, two emissions arising from excitonic and trapped luminescence can be observed. The bands were detected at 428 and 418 nm, assigned to a sulfur vacancy and interstitial sulfur lattice defects. The emission ranges adequately for the optical filter in microscopic detection.

The XPS spectra of core/shell ZnS magnetic nanoparticles characterized the surface elements and structure changes. The binding energy of C_{1s} at 285 eV in polypropylene was used as the reference. The binding energy of $Zn_{2p3/2}$ after zinc ions adsorbed onto the nanoparticles lies at 1022.3 eV, however, the peak of final particles shifts to 1023.9 eV, supporting the formation of zinc sulphide in comparison with zinc peaks. Other peaks of elements were detected, demonstrating the formation of proper structures.

Preliminary protein adsorption test illustrated that less fibrinogen and albumin adsorption on the SPC-protected nanoparticles than that on the pure magnetite. It was clear that the pure magnetite adsorbed protein in few seconds since they precipitate as soon as meeting the injected proteins. Still, the PC-ended magnetite solution remained clear. SDS-PAGE analysis found the nanoparticles remained on the loading zones but separation with albumin during electrophoresis, indicating the weak interaction existence between proteins and nanoparticles. HPLC analysis also verified the similar phenomenon by elution of proteins and nanoparticles. PC-protected nanoparticles weakly adsorbed with albumin and dispersed in protein particles. Notably the supernatant after magnetic separation mainly contained proteins and trace of nanoparticles, while the protein-magnetite (bare) complex particles aggregated being unable to pass through the guarded column. Albumin mixed with certain molar ratios of nanoaprticles solution was eluted form the C8 column showing that proteins should not bound with nanoparticles strongly because of the separated doublet peaks: 1.3 min of retention time for proteins and 1.8 min for nanoparticles (Figure 4). Besides, the MTT activity for the 3T3 cells cultured with nanoparticles demonstrated no significant difference from that for the control. Optical microscope also proved that 3T3 cells sustained increasingly and actively during 24 hr of incubation.

Cell uptake tests were carried out to evaluate the ease of internalization into cells for dispersed nanoparticles. Figure 5 shows the confocal microscopic photographs for the nanoparticles and cells, illustrating a homogeneous distribution of nanopaticles in cell internal. As the literature pointed out that the water-soluble QDs cannot label cells at 4 ℃ after more than 6 hr incubation due to block of endocytosis [4]. Interestingly, the PC-protected nanoparticles were internalized at 4 ℃ for 10 min of incubation, showing another pathway (not endocytosis) such as fusion or diffusion effect participated.

In summary, we have prepared the magnetite core coated with nano-graded ZnS shell to display superparamagnetic and photoluminescent properties. By adsorbing the phosphorylcholine-ended thiol to stabilize the core-shell nanoparticles, the particles can be well-dispersed in aqueous buffer solution for a long term through reducing the interaction with proteins. Furthermore, the particles showed a weak interaction to albumin and a potential cell-labeling uses.

REFERENCES

[1] Y. Zhang, N Kohler, M. Zhang, Biomaterials 23, 1553, 2002.

[2] N. Arul Dhas, A. Zaban, and A. Gedanken. Chem. Mater. 11, 806, 1999.

[3] Y. C. Chung, Y. H. Chiu, Y. W. Wu, Y. T. Tao, Biomaterials 26, 2313, 2005.

[4] J. K. Jaiswal, H. Mattoussi, J. M. Mauro, S. M. Simon, Nature Biotech. 21, 47, 2003.

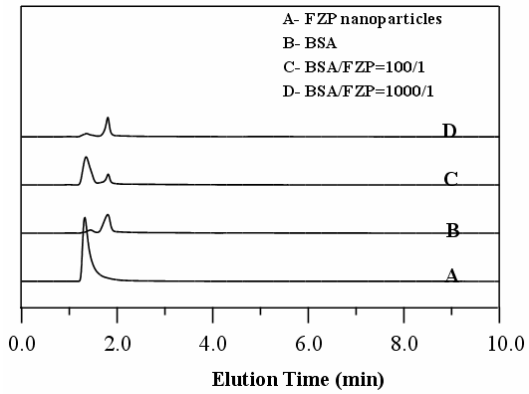

Fig. 4. Elution chart for the separation of proteins and nanoparticles. Curve A for pure PC-protected nanoparticles and B for pure albumin, showing different polarity for these two colloids. Curve C and D based on two mass ratios of albumin/nanoparticles loaded show separated peaks.

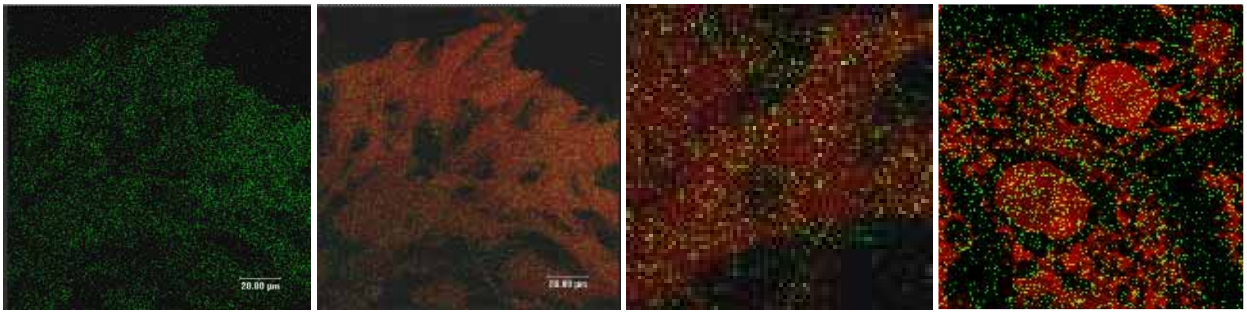

Fig. 5. Micrograph of multifunctional nanoparticles (green, Fig. A) internalized by 3T3 fibroblasts (for 24 hr) and observed by the excitation of 351 nm of UV laser, in comparison with that of cells through autofluorescence (red). Figure B is an overlap of these two images to locate the naoparticles. A close look at the focal plane on the middle part of a cell by photo-section, shown in Fig. C, illustrating the internalization of particles. Figure D shows the diffusion of particles in cells that the pathway of endocytosis was suppressed at 4 ^{o}C for 10 min.