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Preparation of Two-dimensional Array of Nanodots by Ferritin Template

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Zinc selenide (ZnSe) nanocrystals were synthesized in the cavity of the apoferritin from horse spleen (HsAFr), and two-dimensional ZnSe-ferritin nanodots were prepared by simple touch method on modified silicon surface. In the synthesis, ZnSe nanocrystals are encapsulated and growth is restricted to the internal dimension of the protein cavity. The obtained nanodots were characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM), and absorption measurements. In addition, the photoluminescence (PL) properties of two-dimensional nanodots were obtained. From the results, it was concluded that ZnSe nanocrystals were successfully synthesized in the core of ferritin and the monolayer of ZnSe-ferritin could be obtained on the surface of silicon wafer. In addition, the arrays on the silicon surface provided the PL emission peak for ZnSe quantum dots(QDs) in ferritin core. Accordingly, the ZnSe-ferritin arrays can be employed as a useful sensor material for PL technique such as fluorescence resonance energy transfer (FRET).

Keywords: biosensor; ferritin; nanodot; photoluminescence; quantum dot

INTRODUCTION

In the last two decades, ordered nanostructures with microscale featured size on the solid surface have generated considerable interests

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owing to their unique electronic, optical, and biological characteristics [1]. There have been numerous applications of these techniques in electronics and bio-chemical researches such as specific detection of biomolecular interaction which is most important for future drug and diagnostic development [2]. Semiconductor nanoparticle quantum dots are luminescent inorganic fluorophores which can be excited with a single light source for multicolor light emission. They are attractive especially in the area of biosensors and biomarkers due to their long-term photo stability and efficient continuous monitoring. In addition, their size and shape can be controlled by the reaction time, temperature, and ligand molecules used for their synthesis. Accordingly, quantum dots have been tested in most biotechnological applications which employ fluorescence, including DNA array, immunofluorescence assays, and cell biology [3]. In general, for quantum dot preparations, II–VI semiconductor nanocrystals have been widely employed [4–6] and especially for biolabeling applications, there has been a great interest in the cadmium chalcogenide (CdS, CdSe and CdTe) nanostructures in the quantum confinement region mainly due to their optical advantages over the commonly used organic fluorophores. These advantages are not only related to their size tunable optical properties but also to their active surfaces and low photodegradation rates [7]. Regarding the inherent toxicity of these systems that may hinder a safe use in *in vivo* application, there is a natural seek for substituting cadmium ions and producing less toxic labeling materials. To obtain the biocompatibility, some recent reports present ZnSe crystals in the quantum confinement region as potential materials in biolabeling procedures [8–11].

In this work, ZnSe nanodots were synthesized in the cavity of the ferritin from horse spleen (HsAFr), where the ferritin is a self assembled iron storage protein with a 6 nm polypeptide cage. The ferritin has been used for the synthesis and confinement of a range of inorganic materials; such as uranyl oxide, manganese oxide, and magnetite [12]. In addition, the two-dimensional ZnSe nanodots were prepared by simple touch method with modification of silicon wafer surface, which can be used as biosensor materials for photoluminescence (PL) technique such as fluorescence resonance energy transfer (FRET). The obtained nanodots were characterized by TEM, SEM and AFM and the PL properties of two-dimensional ZnSe nanodots and the ferritin nanocrystal solutions were obtained. In any case, the nanoparticles are encapsulated and growth is restricted to the internal dimension of the protein cavity.

EXPERIMENTAL

ZnSe Introduction into the Apoferritin Cavity

The horse spleen apoferritin (HsAFr) was used as received with no further purification. All chemicals were purchased from Sigma-Aldrich. ZnSe introduction into the apoferritin cavity was based on a method described by Ichiro Yamashita [13]. The 5-mL reaction mixture solution with a final concentration of 0.6 mg/mL HsAFr, 2 mM zinc acetate, 80 mM ammonium acetate, and 10 mM selenourea was prepared, and ammonia water was used to maintain the solution at pH 8.2. The addition of selenourea was the final step. Selenourea was dissolved in pure water with a minimal amount of ethanol just before use and added to the reaction mixture solution with a final concentration of 20 mM. The solution was left overnight at room temperature to ensure that the reaction goes to completion. The solution after ZnSe NP synthesis was centrifuged at low speed to remove the precipitates and concentrated by the centrifugal filter. The Sephadex-G25 gel filtration column and 20 nm filter were also applied to remove the impurities as necessary. The protein concentration of the supernatant was measured by the Bradford protein assay method.

Fabrication of the Two-Dimensional ZnSe-Cored Ferritin Array on Si Substrate

Two-dimensional arrays of ferritin molecules were obtained using the method developed by Nagayama *et al*. [14,15] with some modifications. The chemical reagents glucose, MOPS, NaCl, and $CdSO_4$ were of the highest obtainable purity and HPLC water was used.

Polished Si wafers cut along the 111 plane were cut into 5×5 mm pieces. Si substrates were washed in an ultrasonic bath, first in acetone and then in ethanol for 20 min each. The substrates were dried with dry nitrogen gas. The Si substrates were cleaned by plasma stripper, and treated in the HMDS (1,1,1,3,3,3 hexamethyl disilazane) atmosphere in vacuum for 2 h to make the surface hydrophobic prior to the transformation of the array of protein molecules.

A volume of 1 mL of the subphase; 10 mM MOPS, 10 mM $CdSO_4$, 2% glucose (w/v), pH 5.8 was filled into a homebuilt circular teflon trough (diameter 15 mm, depth 5 mm). Then the solution containing 3 μg ZnSe-cored ferritin was injected underneath the dense glucose subphase. Two-dimensional array of ZnSe-cored ferritin was obtained after an incubation period of more than 10 min. The array of ferritin molecules was transferred onto the hydrophobic Si surface by laying

the Si substrate on the air-water interface, which is similar to the Langmuir-Blodgett method. The transferred array was rinsed with HPLC water and excess liquid on the substrate was removed by centrifugation at 5000–10,000 g for 10 s and then stored in a desiccator until use.

Characterization of Two-dimensional Array of ZnSe-Ferritin

UV-Vis spectra were obtained on a diode array spectrophotometer (HP 8453) and the image of ZnSe-ferritin nanodots on silicon surface was observed by SEM (Hitachi S-4700). The AFM image was acquired in non-contact mode using a PSIA Xe-150 (Korea). Silicon cantilevers were used and the AFM scanner and position sensors were calibrated using standard samples from Mikromash. For measuring photoluminescence spectra,a photofluorometer (PTI QuantaMaster^{TM}) was employed.

RESULT AND DISCUSSION

Synthesis of ZnSe Nanocrystal in Ferritin

The synthesis of ZnSe could be monitored by the color change of the reaction mixture solution. That color was transparent at the beginning and changed to light reddish pink after several hours due to the formation of ZnSe nanocrystals. In order to confirm the existence of the nanocrystals, the absorption spectrum was obtained for the pure apo-ferritin and ZnSe-ferritin. As shown in Figure 1, the absorption spectrum provided the characteristic peak of ZnSe nanocrystals at near 380 nm [16], while the peak for pure apo-ferritin showed the corresponding peak at 280 nm [17]. Accordingly, it was concluded that the ZnSe nanoparticles were formed in the core of the apoferritin.

Preparation of the Two-Dimensional Array of ZnSe-Ferritin Nanodots

After preparing the array, SEM and AFM images of the surface of silicon wafer were obtained. In Figure 2, SEM image was given and nanodots of near 14 nm in diameter could be observed. On the other hand, the AFM image in Figure 3 provided the topography for ZnSe-ferritin nanodots on the silicon surface. As shown in the figure, the uniform nanodots were prepared and the result revealed that the average height of the dots was near 10 nm. In reality, the size of the ferritin is about 13 nm and the core of the ferritin is near 6 nm [18].

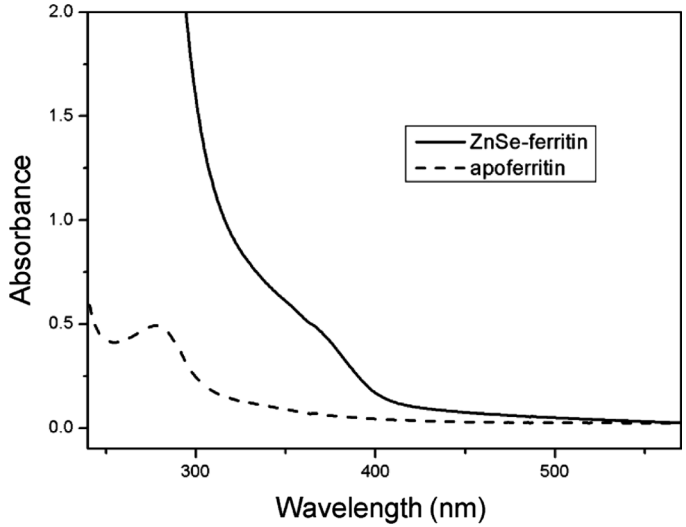

FIGURE 1 Absorption spectra for apoferritin and ZnSe-ferritin.

Accordingly, it could be concluded that the array of ferritin-ZnSe nanodots on the silicon wafer surface was monolayer since the average size of nanoparticles in SEM image and the average height of the nanodots in AFM image were actually same with the diameter of a ferritin.

In Fig. 4, the photoluminescence spectra of the apoferritin and the ZnSe-ferritin in aqueous solutions were given. The excitation

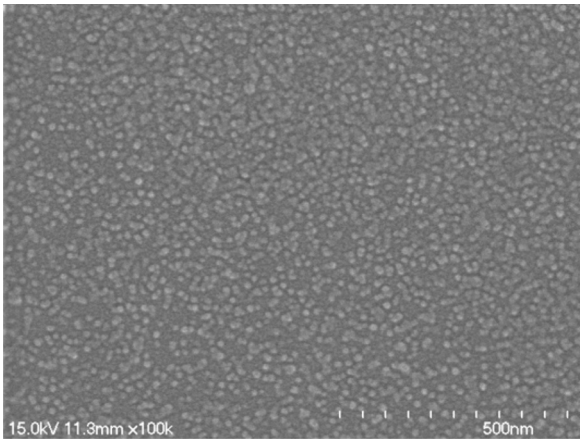

FIGURE 2 SEM image for two-dimensional array of ZnSe-ferritin nanodots on modified silicon surface.

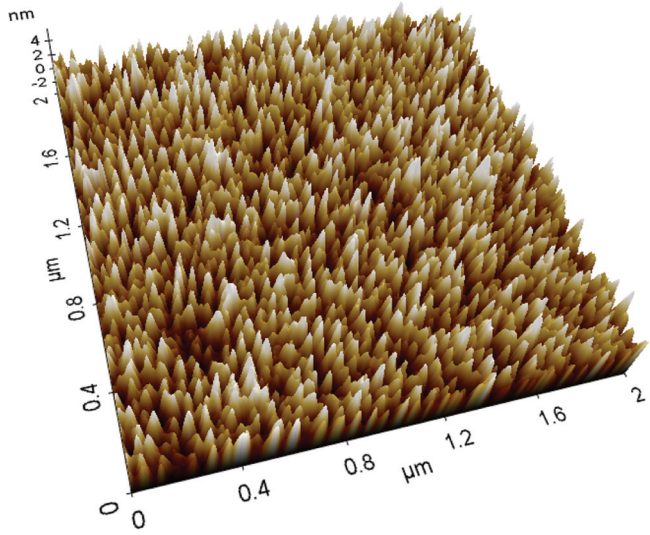

FIGURE 3 AFM image for two-dimensional array of ZnSe-ferritin nanodots on modified silicon surface.

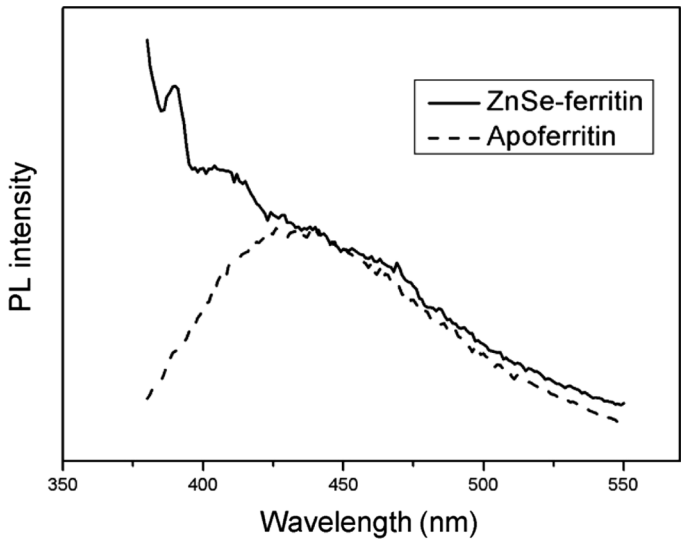

FIGURE 4 Photoluminescence spectra for apoferritin and ZnSe-ferritin solutions.

wavelength was 350 nm and the measurements were performed at 25°C. The results clearly showed that the spectrum for ZnSe-ferritin was different from that for apoferritin due to the existence of ZnSe nanocrystals in the core of ferritin. In addition, the characteristic peak for ZnSe was observed at 375 nm which is consistent with the PL peak for ZnSe of the previous work [16]. Accordingly, from the results of SEM, AFM and PL spectrum, it was concluded that the ZnSe nanocrystals were successfully synthesized in the core of ferretin and the monolayer of the ZnSe-ferritin could be obtained on the surface of silicon wafer.

In reality, the ultimate goal of the present work is preparation of the biosensor material especially for the FRET technique. Fluorescence resonance energy transfer (FRET) is a process via a through-space dipole–dipole interaction between the donor and acceptor pair, which occurs when there is appreciable overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor [19]. The previous workers showed that quantum dots, such as CdTe and CdSe/ZnS, can participate in FRET processes [20,21], which could over come some of the limitations of conventional organic dyes in FRET-based studies of bio-molecules. In order to utilize the ZnSe-ferritin nanodots as a FRET sensor material on solid surface, the ZnSe quantum dots should be fluorescent even in the core of ferritin. Accordingly, the PL spectrum was obtained for ZnSe-ferritin on the silicon surface, and the results are given in Figure 5. The sample was placed into a front face solid sample holder and the sample surface was oriented at 45 deg relative to the incident light. The excitation wavelength was 280 nm and a 320-nm cut-off (long pass) filter was used on the emission side. The strong emission peak at 397 nm for ZnSe quantum dot in ferritn core was observed and the peak was consistent with the previous results. Therefore, the arrays are able to participate in FRET processes. This result is important since the quantum dot with the ferritin shell has some advantages when employed in a FRET system. First, ferritin is biocompatible which is useful property as biosensor material especially for *in vivo* test. Second, the ferritin shells do not allow the aggregation of quantum dots. Thus it is easy to prepare monolayers on solid substrates. Third, in the cavity of the ferritin, the size of quantum dot can be controlled with different reaction conditions. Finally, the thickness of the ferritin shell is thin enough for FRET process. In fact, traditional fluorescence resonance energy transfer (FRET) is efficient for separation distances up to 10 nm [22]. The thickness of ferritin shell is about 3.5 nm. Thus, it could be expected that the ZnSe-ferritin arrays can be used as a biosensor with FRET process.

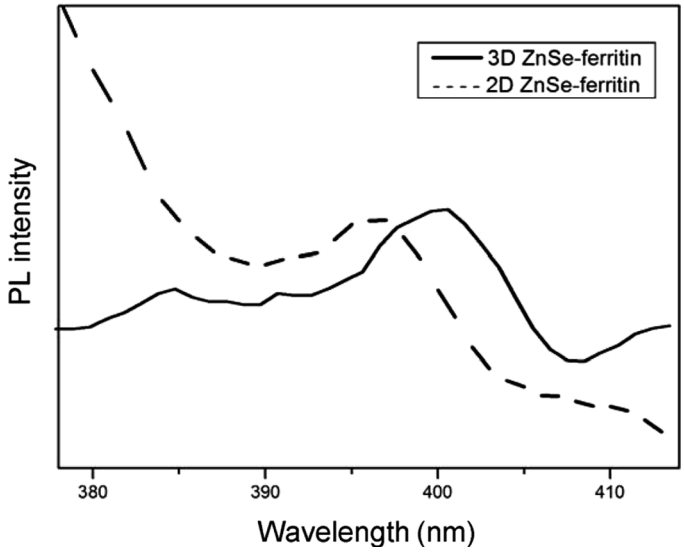

FIGURE 5 Photoluminescence spectra for two-dimensional array and three-dimensional ZnSe-ferritin nanodots on modified silicon surface.

In addition, the PL spectrum for three-dimensional nanodots of ZnSe-ferritin was obtained to compare with those for ZnSe-ferritin two-dimensional array and for ZnSe-ferritin solution. The measuring conditions were the same as that of ZnSe-ferritin two-dimensional array. Briefly, the ZnSe-ferritin solution was dropped on the modified silicon surface and dried in the air to get three-dimensional nanodots. As shown in Figure 5, the characteristic peak for ZnSe was observed at 399 nm. In other words, a PL red shift was observed as the ZnSe-ferritin nanodots were transferred from solution onto the modified silicon surface. Moreover, the red shift for three-dimensional nanodots on the silicon surface was slightly bigger than that for two-dimensional monolayer. This phenomena could be attributed to the energy transfer between semiconductor QDs. Smaller dots in the excited state can transfer their excitation energy to nearby larger dots in the ground state. The efficiency of this process is strongly dependent on the donor-acceptor distance [23]. In addition, it has been proposed that shortening the distance in fluorphore molecules by increasing the fluorphore concentration may cause red-shift phenomena in PL spectra [24]. After the ZnSe-ferritin array was assembled and transferred from solution onto the silicon surface, the average inter-QD distance becomes much shorter. Accordingly, it appears

that the characteristic peak for ZnSe-ferritin solution was red-shifted when the nanodots form an assembled array or three-dimensional nanodots.

CONCLUSIONS

ZnSe nanocrystals were synthesized by employing ferritin template and the two-dimensional ZnSe-ferritin nanodots were prepared by simple touch method with modification of silicon wafer surface. From the results of SEM, AFM, PL and absorption measurements, it was concluded that the ZnSe nanocrystals were successfully synthesized in the core of ferretin and the monolayer of the ZnSe-ferritin could be obtained on the surface of silicon wafer. In addition, the arrays on the silicon surface provided the PL emission peak for ZnSe quantum dots in ferritin core. Accordingly, the ZnSe-ferritin arrays can be employed as a useful sensor material for PL technique such as fluorescence resonance energy transfer (FRET).

REFERENCES

[1] Na, Y. J., Park, S. J., Lee, S. W., & Kim, J. S. (2008). *Ultramicroscopy*, *108*, 1297.
[2] Malavé, A., Tewes, M., Gronewold, T., & Löhndorf, M. (2005). *Microelectron. Eng.*, *78*, 587.
[3] Alivisatos, A. P. (2004). *Nat. Biotechnol.*, *22*, 47.
[4] Nirmal, M., & Brus, L. (1999). *Acc. Chem. Res.*, *32*, 407.
[5] Trindade, T., O'Brien, P., & Pickett, N. L. (2001). *Chem. Mater.*, *13*, 3843.
[6] Bailey, R. E., Smith, A. M., & Nie, S. (2004). *Physica E*, *25*, 1.
[7] Andrade, J. J., Brasil, A. G. Jr., Farias, P. M. A., Fontes, A., & Santos, B. S. (2008). *Microelectron. J.*, *40*, 641.
[8] Nikesh, V. V., Lad, A. D., Kimura, S., Nozaki, S., & Mahamuni, S. (2006). *J. Appl. Phys.*, *100*, 113520.
[9] Geng, J., Liu, B., Xu, L., Hu, F. N., & Zhu, J. J. (2007). *Langmuir*, *23*, 10286.
[10] Shavel, A., Gaponik, N., & Eychmüller, A. (2008). *Microchim. Acta*, *160*, 327.
[11] Geng, J., Liu, B., Xu, L., Hu, F. N., & Zhu, J. J. (2007). *Langmuir*, *23*, 10286.
[12] Wong, K. K. W. & Mayes, E. L. (2003). *Mat. Res. Soc. Symp. Proc.*, *737*, E15.3.1.
[13] Iwahori, K., Yoshizawa, K., Muraoka, M., & Yamashita, I. (2005). *Inorg. Chem.*, *44*, 6393.
[14] Yoshimura, H., Scheybani, T., Baumeister, W., & Nagayama, K. (1994). *Langmuir*, *10*, 3290.
[15] Scheybani, T., Yoshimura, H., Baumeister, W., & Nagayama, K. (1996). *Langmuir*, *12*, 431.
[16] Reiss, P. (2007). *New J. Chem.*, *31*, 1843.
[17] Kim, J., Choi, S. H., Lillehei, P. T., Chu, S. H., King, G. C., & Watt, G G. (2005). *Chem. Commun.*, 4101.
[18] Furuno, T. (2000). *Jpn. J. Appl. Phys.*, *39*, 6435.
[19] Yanga, D., Xua, S., Chenc, Q., & Wang, Y. (2008). *Colloid Surf. A*, *329*, 38.
[20] Shi, L. F., Rosenzweig, N., & Rosenzweig, Z. (2007). *Anal. Chem.*, *79*, 208.

[21] Zhang, C. Y. & Johnson, L. W. (2006). *Anal. Chem.*, *78*, 5532.
[22] Yun, C. S., Javier, A., Nings, T., Fisher, M., Hira, S., Peterson, S., Hopkins, B., Reich, N. O., & Strouse, G. F. (2005). *J. Am. Chem. Soc.*, *127*, 3115.
[23] Wuister, S. F., Swart, I., van Driel, F., Hicky, S. G., & de Donega, C. M. (2003). *Nano Lett.*, *3*, 503.
[24] Shumei, G., Ying, L., Jian, L., & Xiaowu, N. (2003). *Acta Photonica Sinica*, *32*, 261.