PROCEEDINGS OF THE INTERNATIONAL CONFERENCE NANOMATERIALS: APPLICATIONS AND PROPERTIES
Vol. 4 No 2, 02NABM02(4pp) (2015)

Fluorescent Glycine-Coated Silver Nanoparticles as Bio-Imaging Agents for the Neural Stem Cells

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(Received 27 May 2015; published online 26 August 2015)

We study experimentally the photoluminescence of small glycine-coated silver nanoparticles and their application as the bio-imaging markers of the neural stem cells. In addition we study nanoparticle's toxic effects on the neural stem cells. Glycine-coated silver nanoparticles were synthesized using a thermal reduction of silver nitrate in a glycine matrix and size-separated via centrifugation. The properties of the nanoparticles were characterized using transmission electron microscopy, extinction and photoluminescence spectroscopy. Our results indicate that the nanoparticles have deleterious effects on the cells and showed an amplified increase in their death rates. In fixed cells the particles penetrate the membranes within an hour and 25 minutes of incubation, but do not penetrate into the body of the cell.

Keywords: silver nanoparticles, stem cells, toxicity, bio-imaging, surface plasmon resonance, photoluminescence

PACS number(s): 78.55.Hx, 78.67.Bf

1. INTRODUCTION

Recent advances in nanobiotechnology have demonstrated nanoparticles (NPs) as potential drug delivery agents and luminescent biological labels for bio-imaging [1,2]. Consequently, the ability to track and image the fate of NPs starting from the systemic down to the sub-cellular level is an essential factor in drug delivery system. In addition, monitoring morphological and functional alterations in the cells due to the therapeutic delivery is also gaining interest [3]. The development of NPs and their bio-imaging applications signifies the need to study, examine, and monitor their toxicity [4]. In this study, we demonstrated the penetration of glycine-coated silver NPs through the membrane of the neural stem cells (NSCs). We found that glycine-coated NPs induce primarily apoptotic response in time-studies. In unison, these NPs were found to inhibit the proliferation of NSCs. Overall, the functional NPs could potentially be used for controlled drug release and fluorescence imaging of biological cells.

The literature indicates that a considerable amount of research has focused on the generation and development of NPs, however the study of silver NPs in bio-imaging of the NSCs is a novel approach. In this study, we establish that NSCs tend toward the 45% increased cell death when treated with silver NPs. Our studies indicate that NPs penetrate through the cell membranes, however not into their body.

The growing research interest in NSCs is driven by their possible application as a therapeutic tool in neurodegenerative disorders caused by the loss of neural cells. NSCs exist in the developing central nervous system and are capable of differentiating into mature neurons and in some cases non-neuronal cells called neuroglia cells [5]. C17 cells (derived from developing mouse cerebellum) are the primary mammalian cell line used in these experiments. C17s are adherent cells and readily undergo apoptosis when environmental conditions are not ideal [6].

Small noble metal NPs have been extensively studied because of their unique optical and electronic properties [7, 8] which lead to their numerous applications in biochemistry and medicine. Namely, metal NPs offer opportunity for advancements in bio-imaging [9], improvement of solar cells [10], information storage [11], and other.

We synthesized silver NPs through the thermal reduction in glycine matrix. The size separation was performed using centrifugation. The success of the synthesis and size separation is supported by the transmission electron microscopy (TEM) and optical extinction and photoluminescence (PL) spectroscopy characterization. The data supporting NPs characterization is presented in the following section.

2. MATERIALS AND METHODS

2.1 Nanoparticle synthesis and characterization

Silver nitrate (100mg) and glycine (1g) were mixed and dissolved in 10 ml of distilled water. The water was then removed by evaporation with a rotoevap in order to prepare the solid phase mixture. Thermal reduction of the solid phase mixture was completed at 445 K. The reduction of silver nitrate to solid silver was indicated as color of the mixture change from white to dark brown. To purify the product we sonicated and then filtered it with 2 micrometer filter. Next, the product was centrifuged with different relative centrifugal forces (500g – 16000g).

The samples containing NPs of different sizes were characterized by TEM to determine shapes and sizes of particles and confirm the efficiency of size separation by centrifugation. The size distribution of selected

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sample is shown in Fig.1. The JEOL JEM-100CX electron microscope was used for the size characterization. Sample which contained smallest NPs of mean diameters of 9 nm was later used in the bio-imaging and toxicity studies. Most of the particles had a spherical shape.

Extinction and emission spectra of the sample are described in the section 3.1.

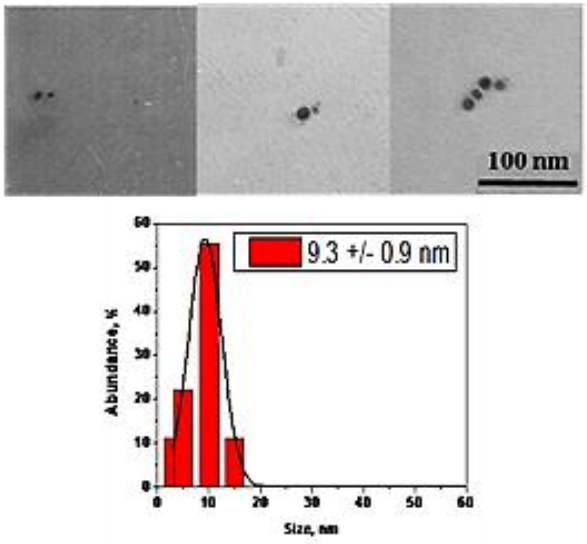


Fig. 1 –TEM of silver nanoparticles of different sizes.

2.2 Cell culture

The C17.2 cell line adopted from the cerebellum of newborn mice are capable of differentiating into neurons and are very sensitive to factors in their surrounding environments. The adherent C17.2 cell line was supplied by Dr. Evan Y. Snyder from Harvard Medical School, Boston, MA. C17.2 neural stem cells (NSC). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) and supplemented with HEPES, Pen/Strep, L-Glutamine, 10% FBS, and 5% Normal Horse Serum. Cells were cultured in normal/unaltered FBS media for five-weeks. Once 2×10^6 to 5×10^6 cells per ml of medium was grown, the cells were lysed and divided. The cells were diluted to a factor of 1:200, and life stage passages 13-18 were used for these studies.

2.3 Cell line treatment

Neural stem cells were isolated and resuspended in the appropriate media. 1×10^6 cells/mL of the cell suspension was used for manual cell counting. The remaining cell suspension was resuspended to *36×10^6 cells per ml*. For viability tests, NSCs were treated with glycine-coated silver NPs and localization tests were performed using 1% paraformaldehyde as the primary fixative to cease all metabolic activities in the NSCs.

3. RESULTS

3.1 Extinction and photoluminescence of silver nanoparticles

Here we present extinction and PL emission spectra of the particles (shown on Fig.2). Extinction spectra (a) reveal surface-plasmon-resonance-related (SPR) peak, situated at 425 nm.

The spectral range of the PL emission (Fig.2 b) coincides with the SPR-related extinction spectral range. It can be rationalized by two theories: first, the interband transitions in silver, enhanced by the SPR, or second, the plasmon emission itself. First theory is based on the idea of the interaction of the photons (emitted via the interband transition of the electron) with the electric field created by the oscillating free electrons (SPR) [12]. Second theory is based on radiative relaxation of the surface plasmon resonance [13].

Peak position in the PL spectrum (Fig.2 b) is slightly red-shifted with respect to the extinction spectral peak (Fig.2 a). It was found at 448 nm. The red shift may be explained by the loss of energy via non-radiative relaxation of the electrons prior to the emission.

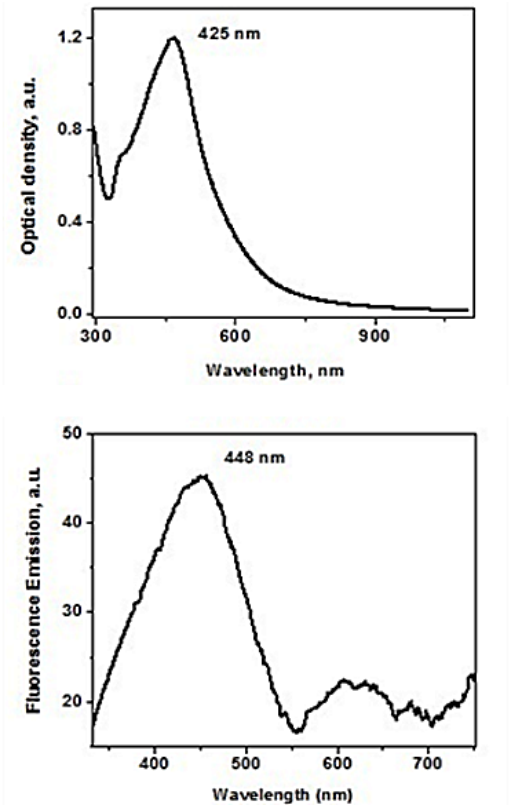


Fig. 2 – (a) Extinction (optical density) and (b) Photoluminescence spectra of nanoparticles of mean diameter of 9 nm.

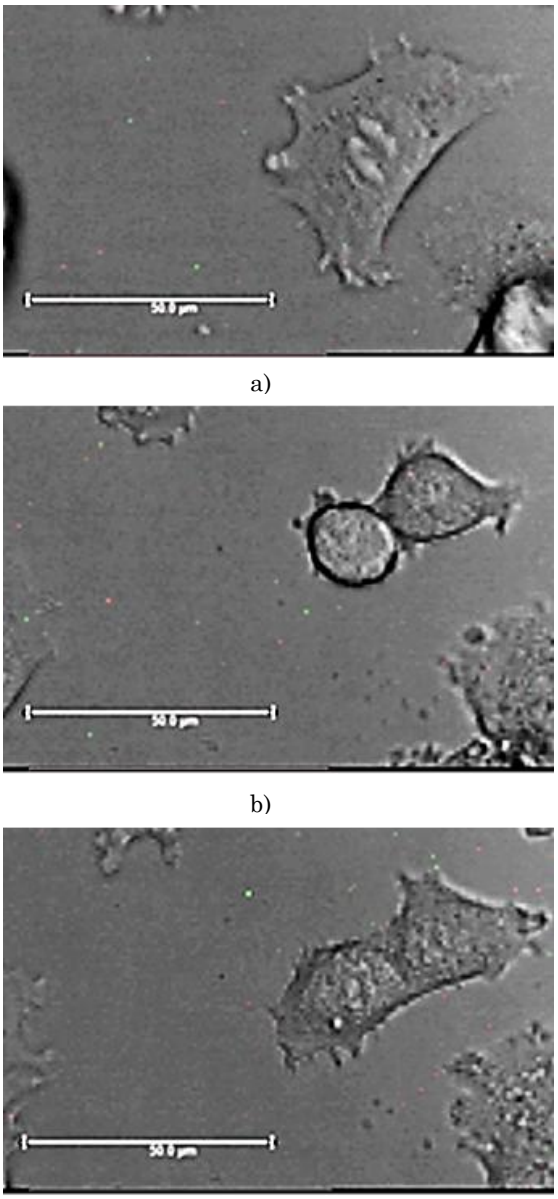
Similarity of the extinction and PL emission spectra illustrates the fact that their mechanisms are related to the SPR.

We picked the sample with the smallest NPs for bio-imaging, because small particles have greater chance to penetrate through the cell membranes. Next section describes the fluorescence confocal microscopy of the neural stem cells incubated with the silver NPs.

3.2 Bio-imaging

We studied penetration of the nanoparticles into the fixed and live cells. As a control we used a sample of cells, without the particles to monitor healthy cells under

preferable conditions. Second sample of cells was kept under the same conditions, but in addition contained nanoparticles. The cells in the control sample were adhered to the well and the cell division could be monitored as shown in Fig. 3.



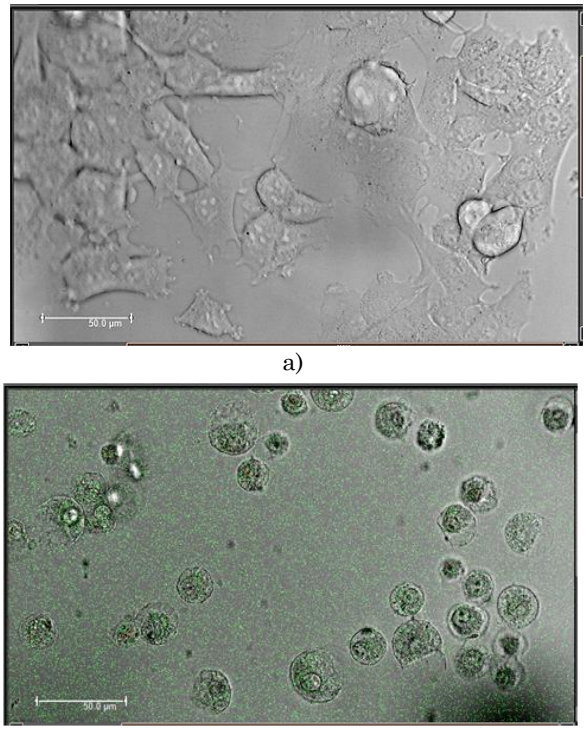
a)

b)

c)

Fig. 3 – Confocal fluorescent and transmission image overlay of the control sample of neural stem cells without NPs. No background fluorescence is present. Cell division can be observed.

Unlike in the control sample (Fig.3), cells in the sample with NPs weren't adhered to the surface of the well shortly after the incubation, they maintained near-spherical shapes during the experiment, and no cell proliferation was observed. The fluorescence signal due to NPs can be seen on Fig. 4b. NPs have caused toxic effect on the cells. The untreated NSCs show about 45% more viable cells than the NP-treated cells. The figure below illustrates the pronounced difference between the blank and the treated NSCs.

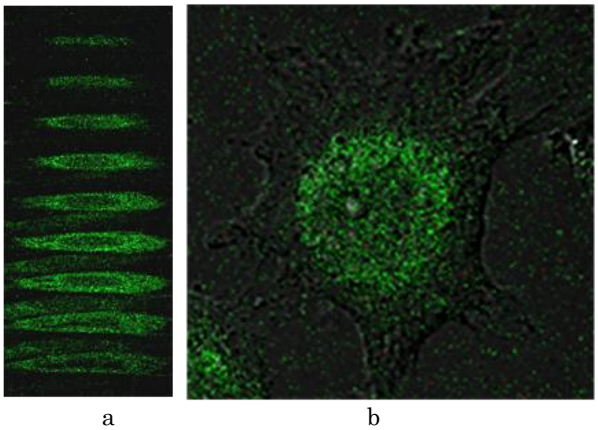


a)

b)

Fig. 4 – Confocal fluorescence and transmission image overlay of the sample of neural stem cells (a) without NPs and (b) with NPs.

We also studied NPs penetration through the cell membranes of the fixed cells in 3D (Fig.5). The experiment was performed over 1 hour 25 minutes. Particles penetrated the cell membrane, but were not observed in the body of the cell (dark area in the center of the Fig.5a). It is possible that longer exposure to the NPs would allow them to go deeper onto the cell body. The so-called "Z-stack" on Fig.5a shows 9 focal planes, obtained within 1 micrometer from each other. The combination of all z-planes together (top view of a cell) is shown on (b).



a b

Fig. 5 – Confocal fluorescence microscopy of (s) Z-stack of the NSC with nanoparticles (a), Top view of all layers collected (b)

4. CONCLUSIONS

We showed that glycine-coated silver nanoparticles of mean diameters of 9 nm have surface-plasmon-

resonance-related photoluminescence. They can penetrate membranes of fixed neural stem cells, and hence can serve as bio-imaging probes in fluorescence microscopy. The particles also cause morphology, proliferation and viability changes to the live cells, mostly causing cell death. This finding potentially can be used for targeted cell destruction.

AKNOWLEDGEMENTS

Authors are grateful to Todd Linden for help with preparation of nanoparticles. We thank to the BioFrontiers Institute and NATO (SFPP-984617) grants for partial financial support.

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