Korean J. Chem. Eng., **30**(1), 235-237 (2013)
DOI: 10.1007/s11814-012-0102-4

Colorimetric detection of vesicle rupture by attack of Ag nanoparticles

Sohyang Shin and Younghun Kim†

Department of Chemical Engineering, Kwangwoon University, Seoul 139-701, Korea
(*Received 24 April 2012 • accepted 30 June 2012*)

Abstract−Generally, the cytotoxicity effects of nanomaterials on bio-organisms have been examined using live cells or mice by *in-vivo* or *in-vitro* testing. These methodologies, however, are time-consuming, and cell-membrane responses for cytotoxicity are often slow. Therefore, in this study, we proposed a simple testing method for colorimetric detection of dye-containing vesicles as biomimetic cell-membranes. When dye-containing vesicles are ruptured by attack of AgNPs (silver nanoparticles), dye in vesicles is released into the solution, and then the color of solution changes to that of dye. Because the ruptured vesicle causes an optical variation, we could easily and quickly monitor the vesicle rupture by AgNPs. And further, SPR (surface plasmon resonance spectroscopy) and TEM (transmission electron microscopy) analysis were carried out to confirm the vesicle rupturing. It will be provide indirect information for cytotoxicity of nanoparticles.

Key words: Dye-contained Vesicle, Silver Nanoparticle, Cytotoxicity Test

INTRODUCTION

Nanomaterials (NMs) have attracted considerable attention in various industries because of their unique physical/chemical properties [1-5]. Specifically, silver nanoparticles (AgNPs) have unique optical and electrical properties, so they have been applied to various fields including electronics, sensors, catalysts, and medical fields [6-8]. It is well known that AgNPs have antibacterial properties and this may be due to their toxicity to other bio-organisms [9,10]. Therefore, cytotoxicity tests for AgNPs are needed for their effective safety management.

The established cytotoxicity testing method for NMs is generally *in-vivo* or *in-vitro* test using live cells or mice. For example, during the toxicity test for the AgNPs using mice, AgNPs were orally administered to mice for 14-24 days [11]. This traditional method, however, is very time-consuming. An alternative testing process needs to be developed for evaluating NMs' cytotoxicity. One of the existing alternative testing methods is *in situ* analysis using surface plasmon resonance (SPR) spectroscopy for cell-membrane rupturing. Because this method requires additional expensive apparatus, we would suggest a simple and directly detectalble methodology for NM cyctotoxicity evaluation.

We prepared the dye-containing vesicles for cytotoxicity testing of AgNPs. Because a dye-containing vesicle has a color, it can be quickly analyzed and easily observed as the structural changes caused by AgNPs occur. After treating with AgNPs, the vesicles will rupture and then dye in vesicles will spread out into the main solution, causing the main solution color to change. Through this color variation, we could evaluate the vesicle rupturing due to AgNPs and quantitatively analyze the cytotoxicity of AgNPs using UV spectroscopy. In addition, we observed the vesicles' morphological variations by SPR spectroscopy and transmission electron microscopy (TEM).

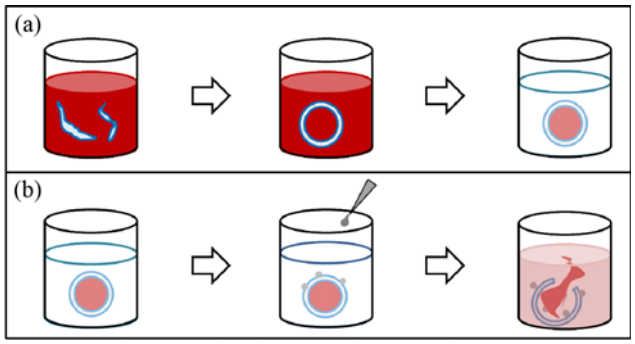
†To whom correspondence should be addressed.
E-mail: korea1@kw.ac.kr

EXPERIMENTAL WORK

Parent vesicles were formed from dimyristoyl phosphatidylglycerol (DMPG, Sigma-Aldrich) in chloroform (Sigma-Aldrich), dried in a stream of N_2; this was followed by removing all traces of the organic solvent in a vacuum oven at 50 °C overnight. Dye-containing vesicles were prepared by the addition of crystal ponceau 6R (acid red, Sigma-Aldrich) in phosphate buffered saline (PBS, Sigma-Aldrich) solution, and kept at 50 °C for about 2 h. Then the solution was centrifuged at 4,000 rpm for 4 min. The samples were kept at room temperature and were used within a few hours after preparation [12]. Silver nanopowder (<100 nm, Sigma-Aldrich) without any stabilzer was dispersed into distilled water using a sonifier at 1 kHz for 1 min (ULH-700S, ULSSO HI-TECH, Korea). The final concentration of the silver powder in distilled water was 400 mg/L, and its average size was about 60 nm. For the AgNPs' cytotoxicity test, 0.73 mM of dye-containing vesicle in PBS solution was treated with 3.7 mM of AgNPs solution (1 : 1 vol%). The rupturing processing was monitored by UV-vis spectroscopy (UV-1800, Shimadzu, Japan) and surface plasmon resonance (SPR) spectroscopy (SPR Lab, K-mac, Korea). The vesicles were immobilized on a gold thin film commercial SPR sensor chip, and then the AgNPs colloidal solution was exposed to the adsorbed vesicles. Spherical vesicles were ruptured locally via attacking AgNPs and then the transformation of the lipid bilayer on the gold chip was induced. Morphological variations of the dye-containing vesicles were observed via TEM (JEM-1010, JEOL, Japan).

RESULTS AND DISCUSSION

The vesicles were generally spherical due to their thermodynamic stablility in solutions [13]. The synthesized vesicles had no color and exhibied the bilayer structure of DMPG, which resembled a cell-membrane. Therefore, the synthesized vesicles could appropriately act as biomimetic cell-membranes and were suitable for cell-



Scheme 1. Schematic diagram of (a) the formation of a dye-containing vesicle, and (b) the rupturing process via attacking AgNPs.

membrane rupturing modeling via attacking AgNPs. The parent vesicles were colorless, so it is not possible to detect the destruction of the bilayer with the naked eye. Therefore, as shown in Scheme 1, we proposed the colorimetric detection method for bilayer rupturing of dye-containing vesicles in solution. The proposed method is very simple and instantly reveals the result of cytotoxicity testing. First, vesicles were prepared in a dye-disolved solution to prepare dye-containing vesicles, which was separated from the free-dye solution by centrifugation followed by dispersion of the dye-containing vesicles in PBS solution.

The color of the prepared dye-containing vesicles was pink, as shown in inset picture of Fig. 1. When a dye-containing vesicle collapses due to the attacking AgNPs, dye spreads out from the vesicle and then its pink color is lost to the solution. If a dye-containing vesicle does not interact with AgNPs, its color will be pink after the filtration and centrifugation steps. Because the PBS buffer's pH is about 7, the charges of the AgNPs and vesicles in the PBS solution are positive and negative, respectively. Therefore, bare-AgNPs were easily bonded to the surface of DMPG-vesicles by the charge-transfer attaction. This feature will affect the structural instability of the vesicles, which induces the vesicle's destruction or rupturing.

Colorimetric detection of ruptured dye-containing vesicles via AgNPs was performed with UV-vis spectroscopy as shown in Fig. 1. Characteristic peaks of acid red in the UV spectra are about 320 and 510 nm, and dye-containing vesicles showed a sholder peak at 510 nm, while AgNP solution has a main absorbance peak at 420 nm. After the addition of AgNPs into the dye-containing vesicle solution, the absorbance peaks changed and showed three peaks at 330, 400, and 510 nm in the UV-vis spectra. As compared with the spectra of dye-containing vesicles, the final solution has two characteristic peaks at 330 and 510 nm, which are almost the same as the peaks of acid red, and one peak at 400 nm, which might be induced by AgNPs. After centrifugation or filtration of the final solution, we obtained a more transparent pink solution. It is noted that the dye-containing vesicles were partially deconstructed by the attacking AgNPs.

The detection process was confirmed with real-time analysis using SPR spectroscopy as shown in Fig. 2. SPR spectroscopy is a very powerful apparatus for the investigation of binding of target materials on a gold chip [16,17]. All processes in the SPR apparatus were continuous flow procedures with buffer solution to wash out overcharged materials on the gold-chip. DMPG vesicles were easily bonded to the gold surface. When dye-containing vesicle solution was exposed to the SPR Au chip (Fig. 2(a)), the reflectance index dramatically increased due to the stacking of vesicles on the gold chip. After 10 min, the value of the reflectance index remained contant. This means that over-stacking vesicles were washed away from the first layer of vesicles on the gold chip, and then the reflectance index stabilized. When vesicles were rinsed with PBS buffer, the reflectance index decreased because weakly attached vesicles were removed (Fig. 2(b)). Then AgNPs were exposed to the gold chip with the vesicles (Fig. 2(c)) and the reflectance index decreased. The inset figure of Fig. 2 shows the changes of the reflectance index of AgNPs coupled with the bare gold surface. After the gold chip was rinsed

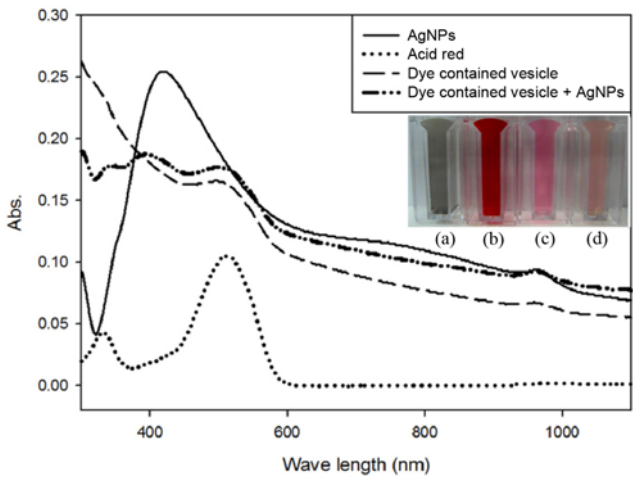


Fig. 1. UV-vis absorbance spectra of the bare AgNPs solution, acid red, dye-containing vesicle solution, and AgNPs-treated dye-containing vesicle. Inset: pictures of each solution; (a) AgNPs solution, (b) acid red, (c) dye-containing vesicle and (d) dye-containing vesicle with AgNPs.

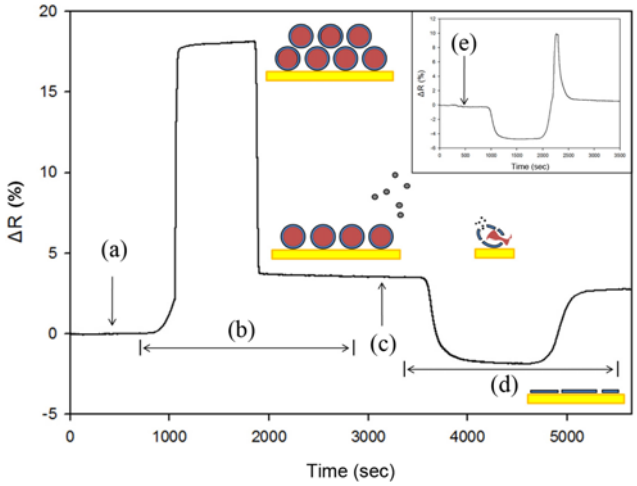


Fig. 2. Time-resolved SPR response curve variation; (a) injection of dye-containing vesicle, (b) attachment of vesicle onto the gold chip surface, (c) and (e) injection of AgNPs, and (d) partially rupturing of the vesicles by AgNPs. Inset: SPR response curve of AgNPs on the gold chip without vesicle.

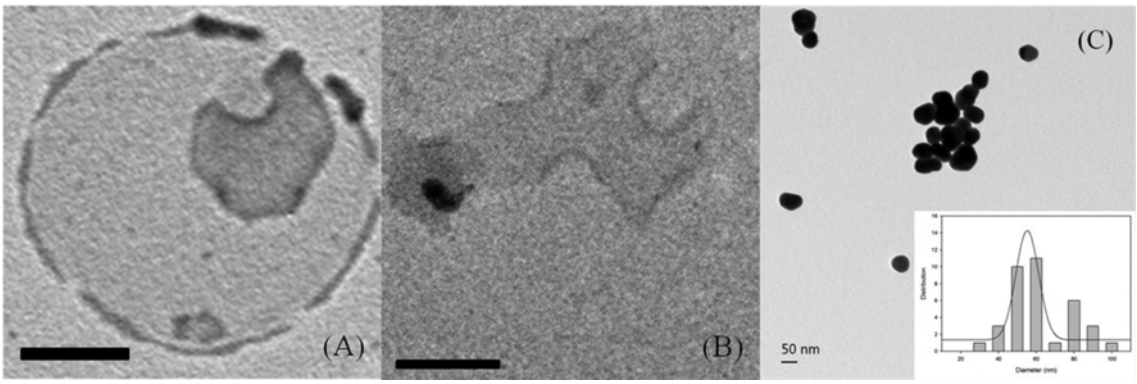


Fig. 3. TEM images of (A) dye-containing vesicle, (B) ruptured vesicle (bar=200 nm), and (C) AgNPs (average size=62 nm). Inset: particles size distribution of AgNPs.

with AgNPs, its reflective index was returned to the original value. This means that the AgNPs did not couple with the bare gold chip without the help of vesicles.

After the injection of AgNPs solution into the dye-containing vesicles immobilized on the gold chip, the reflectance decreased, which was the same as the inset figure of Fig. 2, and then finally a decreased reflectance value was exhibited. It should be noted that partially ruptured dye-containing vesicles were washed out by the rinsing step and the remaining vesicles loaded onto the gold chip represented the decreased reflectance value. This SPR analysis was consistent with the UV-vis analysis.

The morphological changes of the vesicles were analyzed with TEM as shown in Fig. 3. As shown in Figure 3A, there is a gray region in the vesicle, and this may be due to dye molecules. After AgNPs attacked the dye-containing vesicles, the vesicles were deconstructed as shown in Fig. 3(B). In Fig. 3(B), we also found about 60 nm of black spots. This can also be seen in Fig. 3(C).

These findings were coherent with the results of in vitro and in vivo cytotoxicity tests of nanoparticles. Surface charge plays a role in toxicity with cationic surface being more toxic than anionic and neutral surfaces which are most biocompatible, due to the affinity of cationic particles to the negatively charged cell-membrane. Therefore, high zeta potential of nanoparticles is the key factor to stabilize nanoparticles in solutions and is likely to reduce the cytotoxicity. However, we should think about this: high stability will make nanoparticles reside in the environment for a long time.

CONCLUSION

We have proposed a vesicle rupturing system for cytotoxicity testing of NPs using dye-containing vesicles. The dye-containing vesicles had the color of the dye, and the vesicle property analysis was easily performed via UV-vis and SPR spectroscopy. In addition, the vesicles were ruptured by AgNPs and the dye in vesicles was spread into solution by AgNPs; therefore, we could observe the cytotoxicity level of AgNPs in real-time. The rupturing process of the dye-containing vesicles caused a color change in the solution, which was quantitatively analyzed by UV-vis spectroscopy. The morphological variations of the vesicles could be analyzed using SPR spectroscopy and TEM. Using the dye-containing vesicles, we would be able to easily and quickly monitor the cytotoxicity of NPs.

ACKNOWLEDGEMENT

This work was supported by a Research Grant of Kwnagwoon University in 2012 and the Basic Science Research Program through the National Research Foundation of Korea (NRF-2010-0007050).

REFERENCES

1. K. L. Kelly, E. Coronado, L. L. Zhao and G. C. Schatz, *J. Phys. Chem. B*, **107**, 668 (2003).
2. A. Helland, M. Scheringer, M. Siegrtist, H. G. Kastenholz, A. Wiek and R. W. Scholz, *Environ. Sci. Technol*., **42**, 640 (2008).
3. D. Pan, Y. Wang, Z. Chen, T. Lou and W. Qin, *Anal. Chem*., **81**, 5088 (2009).
4. M. Cao, X. He, J. Chen and C. Hu, *Cryst. Growth Des*., **7**, 170 (2007).
5. W.-S. Chae, S.-W. Lee and Y.-R. Kim, *Chem. Mater*., **17**, 3072 (2005).
6. J. Kreuter, *Adv. Drug Deliv. Rev*., **47**, 65 (2001).
7. W. J. Parak, *Nanotechnology*, **14**, R15 (2003).
8. Y. Kim and B. Lee, *Korean Chem. Eng. Res*., **49**, 393 (2011).
9. E. Bae, H.-J. Park, J. Yoon, Y. Kim, K. Choi and J. Yi, *Korean J. Chem. Eng*., **28**, 267 (2011).
10. K. A. Riske, H.-G. Döbereiner and M. T. Lamy-Freund, *J. Phys. Chem. B*, **106**, 239 (2002).
11. E.-J. Park, E. Bae, J. Yi, Y. Kim, K. Choi, S. H. Lee, J. Yoon, B. C. Lee and K. Park, *Environ. Toxicol. Phar*., **30**, 162 (2010).
12. N. Lubick, *Environ. Sci. Technol*., **42**, 8617 (2008).
13. K. A. Riske, L. Q. Amaral and M. T. Lamy, *Langmuir*, **25**, 10083 (2009).
14. Y. Wang, A. Neyman, E. Arkhangelsky, V. Gitis, L. Meshi and I. A. Weinstock, *J. Am. Chem. Soc*., **131**, 17412 (2009).
15. T. Wang, D. Zhang, W. Xu, J. Yang, R. Han and D. Zhu, *Langmuir*, **18**, 1840 (2002).
15. T. L. Williams, M. M. L. M. Vareiro and A. T. A. Jenkins, *Langmuir*, **22**, 6473 (2006).
16. L. Zhang, M. L. Longo and P. Stroeve, *Langmuir*, **16**, 5093 (2000).