Functionalized Silver Nanowires for Live Cell Study

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The biocompatibility of the surface-functionalized silver nanowires has been investigated in two cell lines. It was found that the functionalized silver nanowires with 200 nm diameter and length up to a few μm can be internalized by cells. The cytotoxicity of the silver nanowires was found to depend on their surface modifications. It was also demonstrated that the surface-enhanced Raman scattering (SERS) could be measured on the silver nanowires, which added additional functionality to the silver nanowires.

To solve complex biological problems, it often requires the most advanced tools and techniques. With recent progress in nanotechnology, many novel nanomaterials have been synthesized to exhibited superior performance to the conventional materials in biosensing[1,2] and biolabeling.[3,4] However, the utilization of these nanomaterials for the investigation of living cells is less explored owing to the concern of biocompatibility and cytotoxicity.[5,6] Metallic nanoparticles, such as gold and silver, have long been used in the biological studies because of their easy preparation process and long-term stability. With the help of nanotechnology, many new biological assays based on metallic nanoparticles have been developed for labeling,[7] drug delivery,[8] and gene regulation.[9] Because of the complexity in biological system, it is always desirable to engineer new materials with as much functionality as possible to extract the maximum amount of useful information in a single assay. For example, the optical signatures of nanoparticles, such as fluorescence or Raman signal, can be used to trace the location of the nanoparticles while the magnetic nanoparticles can be used to manipulate the cells through external magnetic field. In addition, the surfaces of nanoparticles can be modified with various biomolecules allowing specific biochemical bindings or reactions.[10]

Among the newly developed nanoparticles, the nanorods or nanowires produced by template electrodeposition are particularly useful because of their simple fabrication procedure and capability for multiplexing. Micrometer long multisegment nanowires have been prepared as barcodes for biological multiplexing.[11] It has been shown that the multisegment nanorods could carry plasmid DNA on one segment while the other segment could be modified with targeting ligands to enhance the uptake of the nanorods.[12] Recently, it has been demonstrated that the micrometer long nickel nanowires could be used to manipulate live cells through magnetic field.[13] Previously, we have investigated the cytotoxicity of the micrometer long gold nanowires and the behavior of nanowires inside live cells as well as the transfection efficiency of the gold nanowires.[14,15] It was found that the cytotoxicity of the gold nanowires depended on their surface modifications and that the gold nanowires could be used as an efficient gene carrier. Silver nanowires are another class of nanomaterials, which exhibit very high surface-enhanced Raman scattering (SERS) cross section, capable of conducting multiplexing assay in live cells. To explore the possibility of using silver nanowires for live cell study, we have investigated the functionality and cytotoxicity of the surface-modified silver nanowires.

To fabricate the silver nanowires, commercial alumina oxide membranes with a diameter of 200 nm (Whatman) were used as the templates. The Cy-less Silver 2 RTU (Technic Inc.) electroplating solution was used for silver nanowire production. The length of the nanowires can be controlled by monitoring the total charge passing through the electrochemical cells and the deposition time. The nanowires were obtained by removing the membrane in 3 M NaOH solution and sonication for several minutes. The nanowires were further cleaned by rinsing in deionized water and centrifuging at 5000 rpm for 10 min several times. The concentration of the nanowire solution was measured by a hemacytometer and observed with 20× objective.

To demonstrate that the SERS signal from the nanowires could be used to label the nanowires, silver nanowires were mixed with 1 mM Rhodamine 6G for 1 min. The SERS spectrum of the Rhodamine 6G molecules on the nanowire surface is shown in Figure 1a where the peaks around 1187, 1315, 1365, 1514, and 1653 cm^{-1} can be attributed to Rhodamine 6G molecules, which indicates that the SERS signal from the nanowires is strong enough for labeling purpose. Therefore, the SERS signal can be used to label the silver nanowires. To further explore the possibility of using silver nanowires as multiplexing assays in live cells, the two segment silver–nickel nanowires[12] were modified with 3-[(2-aminoethyl)dithio]propionic acid (AEDP), which allowed the binding of plasmid DNA molecules on the nickel segment through electrostatic interaction and the manipulation of nanowire through magnetic field. The combined fluorescence and DIC (differential interference contrast) image of the nickel–silver nanowires inside a fibroblast cell was illustrated in Figure 1b. As seen from Figure 1b, the silver–nickel nanowires were internalized by the fibroblast cells. The green fluorescence signal in Figure 1b was coming from the YOYO-1 dye,

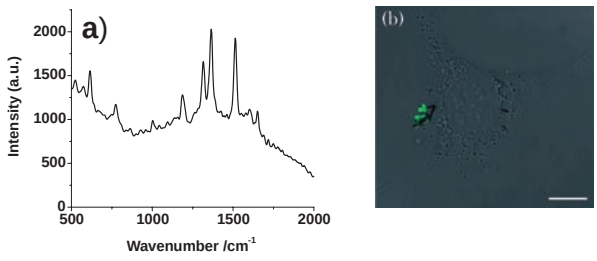

Figure 1. a) SERS signal from the Rhodamine 6G molecules on the silver nanowires. b) Silver–nickel nanowires internalized by a fibroblast cell. Green color is the plasmid DNA on the nickel segment labeled by YOYO-1 dye. Bar: 10 μm.

which was used to label the plasmid DNA on the nickel segment, indicating that the micrometer long silver–nickel nanowires could carry plasmid DNA molecules into the fibroblast cells.

Knowing that silver nanowires could deliver plasmid DNA into cells and that the SERS signal from the silver nanowires could be used to label the nanowires, it is very important to investigate the cytotoxicity of various surface-functionalized silver nanowires, if the silver nanowires are going to be used for the live cell study. To produce silver nanowires with different surface modifications, the suspended silver nanowire (1×10^6 nanowire/mL) solutions were mixed with an ethanol solution of 1 mM of 11-amino-1-undecanethiol (Dojindo), 1 mM of octadecanethiol (Aldrich), or 1 mM of 11-mercaptoundecanoic acid (Aldrich). The aminothiol-modified nanowires exhibited positively charged surfaces whereas the negatively charged surfaces were obtained by modifying the nanowires with mercapto acids. Alkanethiols were also used to prepare nanowires with neutral surfaces. Since the serum could attach to the bare nanowire surface, we also tested the serum-coated nanowires. The cytotoxicity of the surface-modified nanowires was tested in two cell lines, Fibroblast HS68 and HeLa S3 (BCRC). The medium used was Dubbeco's modified Eagle's medium (DMEM, Gibco) for fibroblast cells and Minimum Essential Medium (MEM, Gibco) for HeLa cells. 10% fetal bovine serum (FBS, PAA Laboratories) and PEN-STREP-AMPHO solution (Biological Industries) were added to both culture media.

To determine the cytotoxicity of the silver nanowires, the cells were first seeded in 96-well plates at a density of 1×10^5 cell/mL at 37 °C in 5% CO_2 atmosphere. After 24 h of incubation, the wells were refilled with the fresh medium and serial dilutions of nanowires with nanowire concentration ranging from 10^3 to 10^6 nanowire/mL. Control experiments were carried out with cells treated with an equivalent volume of serum medium without any nanowires. Cells were then incubated for 24 h at 37 °C. Cytotoxicity was measured using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to measure the succinate dehydrogenase mitochondrial activity. PBS solutions containing 10 μL of 5 mg/mL MTT stain (in vitro toxicology kit, Sigma) were added into each well and incubated for 2 h. The absorbance in each well was measured at 570 nm in a microplate reader (μQuant, Biotek Instrument). Background absorbance was measured in PBS solution without the presence of cells and nanowires. All experiments were repeated three to nine times.

In a typical viability test, 5 μm long silver nanowires with various surface modifications were used. After 24 h incubation of the cells, it was found that the cytotoxicity of the silver nanowires increased as the density of the nanowires increased and that all surfaces-modified nanowires except the serum-coated nanowires exhibited some degree of toxicity to both cell lines similar to those observed in the presence of the gold nanowires.[15] However, in contrast to the gold nanowires, the neutral silver nanowires were found to more toxic in the fibroblast cells, and the negatively charged silver nanowires were found to be less toxic for both cell lines. The results of the cell viability in the presence of silver nanowires with different coatings in two cell lines are summarized in Figure 2. In general, the cytotoxicity for the silver nanowires was lower than the cytotoxicity for the gold nanowires.[15] The origin of this discrepancy is not known at this moment.

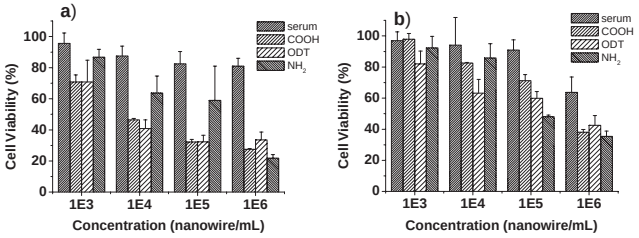

Figure 2. Cytotoxicity for a) fibroblast and b) HeLa cells in the presence of various surface-functionalized silver nanowires.

In summary, we have tested the cytotoxicity of the silver nanowires with four different coatings, serum, alkanethiols, mercapto acids, and aminoalkylthiols, in two cell lines. The serum-coated silver nanowires were found to be the least toxic whereas the aminothiol-modified sliver nanowires were shown to exhibit moderate toxicity and were used to deliver plasmid DNA molecules into cells. It was also shown that the SERS signal from the silver nanowires could be detected. Therefore, we conclude that it is possible to develop live cell multiplexing assays using silver nanowires.

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