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The Applications of Metallic Nanowires for Live Cell Studies

Chiung-Wen Kuo and Peilin Chen

*Research Center for Applied Sciences, Academia Sinica
Taiwan*

1. Introduction

Recently, there has been increasing research attentions focused on the development of nanomaterials for solving complicated biological problems. Nanoparticles, such as quantum dots or metallic nanoparticles, have been shown to exhibit superior performance to the conventional techniques in biosensing (Nam et al., 2003; Xiao et al., 2003) and biolabeling (Michalet et al., 2005; Medintz et al., 2005). However, the applications of nanoparticles for the studies of living cells are less explored due to the issues of biocompatibility and cytotoxicity (Derfus et al., 2004; Goodman et al., 2004; Chithrani et al., 2006). Noble metals, such as gold, have been used in the biological studies for a long time because of their stability and low toxicity. The use of metallic nanoparticles may offer several advantages in biomedical applications including simple preparation, well-defined size, various available surface modification schemes, and high sensitivity detection. Therefore, there are renewed research efforts in developing metallic nanoparticle based techniques for labeling (Katz & Willner, 2004), drug delivery (Shen et al., 2004; Salem et al., 2003; Sandhu et al., 2002) and gene regulation (Rosi et al., 2006).

A common approach to use nanomaterials for biomedical applications is to chemically modify the surfaces of the nanoparticles such that the nanoparticles can recognize a specific molecule or receptor on the cell surfaces or the nanoparticles can form complexes with drugs or genetic materials to enter the cells. However, in the complicated cellular environments, it often requires individual nanoparticles to possess several functionalities to achieve multiple tasks. The surfaces of nanoparticles may have to be decorated with biomolecules to recognize specific cells or to enhance the uptake efficiency. When the nanoparticles are inside the cells, additional molecules may be needed to help the nanoparticles to escape the endosomes or to reach specific organelles. In addition, the optical properties of nanoparticles may allow monitoring the cellular uptake process and their spatial distribution by optical microscope whereas the magnetic nanoparticles may be used for separation or contrast agent. To engineer the nanoparticles with multiple functionalities, two peptides have been attached to the same gold nanoparticles allowing traversing cell membrane by receptor-mediated endocytosis pathway and endosomal escape (Tkachenko et al., 2003). However, these two peptides were randomly distributed on the surfaces of nanoparticles. It is very difficult to control the spatial distribution of molecules or functionalities on the spherical nanoparticles. This problem can be solved by using non-

Source: Electrodeposited Nanowires and Their Applications, Book edited by: Nicoleta Lupu,
ISBN 978-953-7619-88-6, pp. 228, February 2010, INTECH, Croatia, downloaded from SCIYO.COM

spherical nanoparticles such as multi-segment nanowires, which could be engineered with different functionalities in a spatial controlled manner through various coupling schemes. It has been shown that two types of molecules have been incorporated onto the gold-nickel nanowires where the gold end was used to bind to a plasmid DNA through electrostatic interaction while the nickel surface was engineered to carry a specific polypeptide for site recognition (Salem et al., 2003). To extend this type of applications, micrometer long metallic nanowires may be useful. Micrometer long multi-segment nanowires have been used as barcodes for biological multiplexing, which could be easily visualized by an optical microscope (Keating & Natan, 2003). The micrometer long nickel nanowires could be internalized by cells allowing the manipulation of living cells through magnetic field (Tanase et al., 2005). However, it is not known whether the micrometer long metallic nanowires can be internalized by the cells without damaging the cells, which is an important issue for the development of nanowires based living cell probing system. If the nanowires can be internalized by the cells, it is possible to observe the intracellular microenvironment around the individual nanowires through an optical microscope. In this chapter, we will focus our discussion on the fabrication and functionalization of metallic nanowires for probing living cells.

2. Synthesis of metallic nanowires

In general, metallic nanowires can be synthesized by two different approaches: chemical synthesis (bottom up) and template deposition (top down). While the chemical synthesis has been used to produce nanowires with various aspect ratios in great quantity, the compositions of nanowires are very limited due to the available precursors and synthesis procedures. On the other hand, template deposition allows not only the fabrication of metallic nanowires with a wide range of compositions but also the synthesis of nanowires with very high aspect ratios. Therefore, the template deposition is widely used in the production of metallic nanowires. To fabricate metallic nanowires, two types of templates are often used: anodic aluminum oxides (AAO) and polycarbonate membranes. The metallic nanowires can be obtained by electro-deposition of the desired materials inside these porous membranes. The back sides of the membranes are coated with a layer of metal, which serves as the working electrode for the electro-deposition. The length of nanowires can be controlled via deposition time and current. The compositions of metallic nanowires can be regulated using various plating solutions resulting in multi-segment nanowires. For the template deposition, the AAO membrane is the most popular one due to their availability and tunable pore size. AAO templates have been widely used for the fabrication of various one dimensional nanomaterials such as polymers (Jessensky et al., 1998; Xu et al., 2007), semiconductors (Pena et al., 2002; Divliansky et al., 2001) metals (Mitchell et al., 2002) using electrochemical deposition. For example, using sequential electrodeposition, multi-component nanowires have been fabricated (Keating & Natan, 2003). Because of the variations in composition along the length, it has been suggested that different surface chemistry could be used to modify individual segment of the nanowires. These functionalized nanowires have been demonstrated capable of conducting chemical and bio-sensing. In another study, the rotation of the multi-segment nanowires in the presence of the hydrogen peroxide was observed due to the catalytic decomposition of hydrogen peroxide on the nickel segment (Fournier-Bidoz et al., 2005). The AAO membrane can be purchased from commercial vendors or prepared in-house by electrochemical etching process. To

prepare AAO templates, thin aluminum sheets are anodized in electrolyte under appropriate anodizing voltage. With different electrolytes such as oxalic acid or sulfuric acid solution and appropriate voltages, it is possible to control the pore size of the AAO membranes. Ordered AAO templates can also be obtained by imprinting an ordered layer of nanostructures on the surfaces and followed by the self-organization process of AAO templates (Masuda & Fukuda, 1995). After the electrodeposition, the AAO templates can be removed by saturated HgCl_2 solution.

3. Surface modifications

To utilize the nanoparticles in the biological system, it often requires surface modifications of the nanoparticles to improve their biocompatibility. In addition, the added functionalities through surface modifications would allow the binding of the nanoparticles to specific biomolecules or carry other molecules such as drugs. For metallic nanoparticles, the surface modification scheme normally involves the formation of self assembled monolayer through thiol, amino, cyanide and carboxylic acid group. Charged or hydrophilic groups are often incorporated into the nanoparticle surfaces to provide solubility in water and functionality for interaction with biomolecules. Hydrophilic groups, such as oligo(ethylene glycol)(OEG) and Poly(ethylene glycol) (PEG) are commonly used on the nanoparticle surfaces. Surface adsorption of proteins will result in denaturing of proteins and, in some cases, may limit the interactions of the ligand with the target on the cell surface due to steric hindrance (You et al., 2007). OEG and PEG are known to resist the non-specific interaction with biomolecules, which in turn improves the biocompatibility of nanoparticles.

As for the surface modification of nanorods or nanowires, it is possible to selectively modify the multi-segment nanorods and nanowires. For example, selective surface modification scheme has been employed to modify two component nickel-gold nanowires to achieve bi-functionality (Bauer et al., 2003). In this case, the nanowire were reacted with 11-aminoundecanoic acid and 1,9-nonanedithiol. It was shown that the carboxylic acid could bind to the nickel segment while the thiol group was used to modify the gold segment surface. The result of dual functionalization could be observed by the fluorescence microscopy (Bauer et al., 2004). Since multi-segment nanowires could exhibit dual functionalities, they can offer simultaneous detection and separation of multiple species in solution. Mirkin et al. have demonstrated that Au-Ni-Au nanowires could be used for target binding and separation of biomolecules process. To achieve this goal, the gold portions of the nanorods were passivated with 11-mercaptoundecyl-tri (ethylene glycol)(PEG-SH) and fluorescein-tagged poly-His (His > 6) was then bound to the Ni portions of the substrate as evidenced by confocal fluorescence microscopy (Lee et al., 2004; Hurst et al., 2006).

To modify the surfaces of nanowires, a simple approach is to functionalize the surfaces of nanowires with amino, methyl and carboxyl terminal group through corresponding thiols. Additional molecules can be attached to these functional group via different coupling schemes. The surface modification of nanowires could be achieved by mixing the suspended gold or silver nanowires (1×10^8 nanowire/ml) solutions with an ethanol solution of 1 mM of 11-amino-1-undecanethiol, 1 mM of octadecanethiol, or 1 mM of 11-mercaptoundecanoic acid. After 24 hours of incubation, the nanowires were cleaned by the doubly distilled water and the excess thiols were removed by dialysis for 24 hours using a 3.5kD cut off dialysis membrane in the doubly distilled water. For comparison, the serum coated nanowires were also investigated where the serum coated nanowires were obtained

by incubating the nanowires in the PBS solution containing serum for 24 hours. All surface modified nanowires can be suspended in aqueous solutions by strong vortexing. However, longer nanowires exhibited higher sedimentation rate. The zeta-potentials of the surface modified nanowires were measured by a Zeta Potential Analyzer at a field of 8-16V/cm.

4. Cytotoxicity of metallic nanowires

There is an increasing trend in using nanomaterials in biomedical applications for the purpose of diagnosis, imaging, and drug delivery. However, it is only until recently that the issues of the toxicity and health effects of the nanoparticles have been widely investigated. In many cases, additional coatings for the nanoparticles are needed to optimize their utility in the cellular studies. For instance, magnetic nanoparticles without polymer coating often suffer from the aggregation in water or tissue fluid, which may limit their applications in isolation and detection. Surface coatings may affect the particle size, physical properties, and the corresponding effects on toxicological properties. For example, it has been reported that the cytotoxicity was not significant for the nickel ferrite nanoparticles with a hydrophilic surface (Yin et al., 2005). However, the cytotoxicity was observed for nickel ferrite nanoparticles modified with a layer of oleic acid. Therefore, it was concluded that the hydrophobic coating was more toxic than the hydrophilic surface. Size is another important factor for cytotoxicity. At higher concentrations, both small and large nanoparticles exhibited similar cytotoxicity. Since the size of the nanoparticles could be related to their surface interaction area and surface energy, it was found larger nanoparticles (about 150 nm) was more toxic than smaller nanoparticles (10 nm). In a separate experiment, it has been shown that the intercellular delivery of anionic iron oxide nanoparticles reduced the ability of PC12 cells to respond to nerve growth factor in a dose dependent manner. The use of anionic magnetic nanoparticles changed cell phenotype and behavior, which could be directly correlated with the level of nanoparticle exposure (Pisanic et al., 2007).

As for the metallic nanowires, the CTAB precursor used in the synthesis process may be the source of the cytotoxicity. (Connor et al., 2005, Takahashi et al., 2006). It has been clearly demonstrated that when the nanowires were coated with phosphatidylcholine molecules, the cytotoxicity was reduced. In another experiment, PEG-modified gold nanorods were used to reduce the nonspecific binding with blood component such as blood proteins and blood cells. For the micrometer long nanowires, we have investigated the cytotoxicity of nanowires with different surface charges for two cell lines, NIH 3T3 fibroblast cells from normal tissue, which is an adherent cell line, the HeLa S3 cells from neoplastic tissue, which can grow in suspension media (Kuo et al., 2007).

To determine the cytotoxicity in the presence of gold nanowires, the cells were first seeded in 96-well plates at a density of 1×10^5 cell/ml at 37°C in 5% CO₂ atmosphere. After 24 hours of culture, the wells were refilled with the fresh medium and serial dilutions of nanowires at nanowire concentration ranging from 10^3 to 10^6 nanowire/ml. 90 µl of the nanowires solutions at different concentrations were added to each well. Control experiments were carried out with cells treated with an equivalent volume of serum medium without any nanowires. Cells were then incubated for 24 hours at 37°C. Cytotoxicity was investigated 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 were added into each well and incubated for 4 hours. After mixing, 90 µl of MTT solubilization solution was added into each well. The stain was aspirated and

the purple color crystal was dissolved with acidic isopropyl alcohol. After 15 minutes, the absorbance in each well was measured at 570 nm in a microplate reader. Background absorbance was measured in PBS solution without the presence of cells and nanowires. All experiments were repeated 3 to 9 times. The cell viability (%) related to the control wells containing the cell culture medium without nanowires was calculated by $[A]_{\text{test}}/[A]_{\text{control}} \times 100$ where $[A]_{\text{test}}$ was the absorbance of the test sample and $[A]_{\text{control}}$ was the absorbance of the control sample.

To investigate the cytotoxicity effect of the surface modifications, the aminothiols were used to produce surfaces with positive charge, and the mercapto acids were used to generate negatively charged surfaces whereas the alkanethiols were used as a nonionic surface modification. For comparison, the nanowires coated with serum were also tested. The viability tests for fibroblast 3T3 and HeLa cells were summarized in figure 1(A) and (B). In a typical viability test, 4.5 μm long gold nanowires with various surface modifications were used. The density for both cells was about 10^5 cell/ml, and the density of the nanowires was varied from 10^3 to 10^6 nanowire/ml. After 24 hours incubation of the cells, it was found that the cytotoxicity increased as the density of the nanowires increased and all surfaces modified nanowires except the serum coated nanowires exhibited some degree of toxicity to both cell lines.

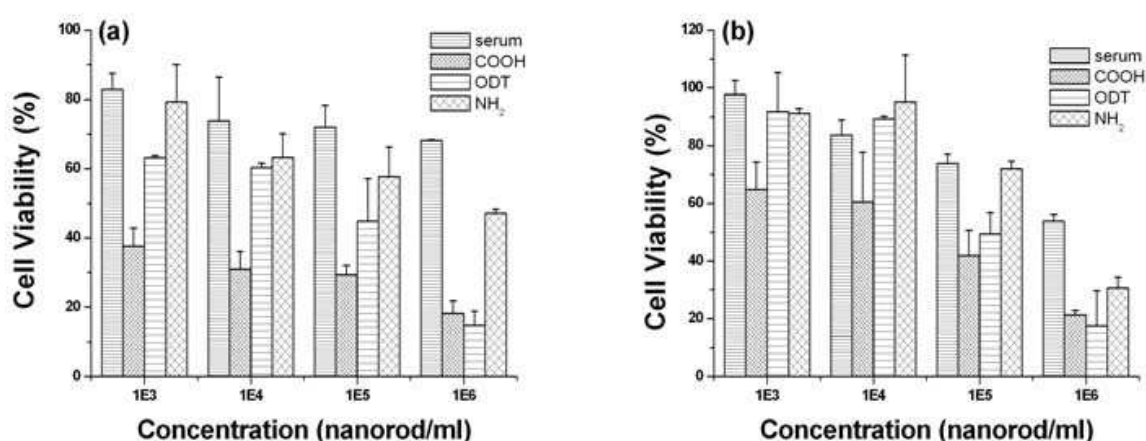


Fig. 1. The cytotoxicity of gold nanowires with various surface modifications for (a) fibroblast cells and (b) HeLa cell.

At a lower density ($< 10^4$ nanowire/ml), most of the cells were unaffected by the addition of the nanowire solution. However, the carboxyl presenting nanowire surfaces exhibited very strong cytotoxicity even at a very low nanowire density (10^3 nanowire/ml). At a higher nanowire density, most of the cells were injured by the addition of nanowire solution except for the serum coated nanowires. Large aggregation of nanowires was observed at a density of 10^7 nanowire/ml, which made it difficult to evaluate the number of nanowires. At a nanowire density higher than 10^8 nanowire/ml, the bottom of the well was completely covered by the nanowires. The LD_{50} value of the serum coated gold nanowires for 3T3 cells was estimated to be 5×10^7 nanowire/ml ($\sim 150 \mu\text{g/ml}$), which was lower than the value measured for smaller gold nanoparticles ($\sim 750 \mu\text{g/ml}$) (Salem et al., 2003). The reason for the lower LD_{50} for the gold nanowires could be attributed to larger size nanowires used in this experiment.

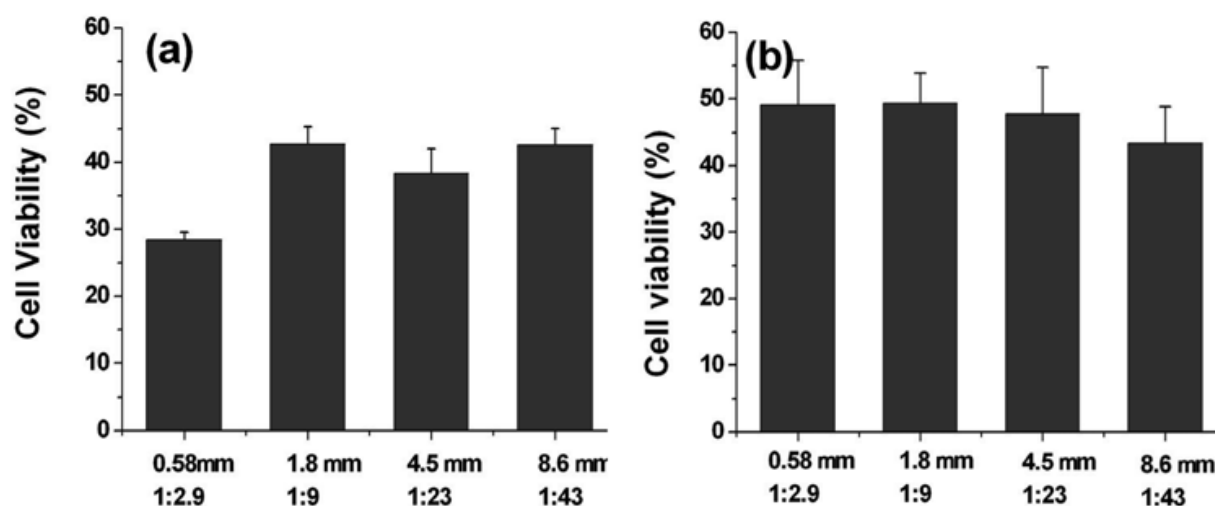


Fig. 2. The cytotoxicity of different sizes of gold nanowires modified with mecapto acid for (a) fibroblast cells (b) HeLa cell.

In a previous study (Chithrani et al., 2006), it was reported that the cellular uptake of gold nanoparticles depended on both the size and the shape of the nanoparticles. With nanoparticle size smaller than 100 nm, it was found that the uptake efficiency of different size of nanoparticles peaked at 50 nm. And it was also concluded that the uptake efficiency decreased as the aspect ratio increased. If the cellular uptake depends on the size of the nanoparticles, the cytotoxicity will also vary with the size of nanoparticles. Therefore, we have investigated the cytotoxicity of different sizes of nanowires with various aspect ratios from 1:2, 1:10, 1:25, and 1:50. In this study, the concentration of mercapto acid modified gold nanowires was 10^5 nanowire/ml for all aspect ratios. The results of cytotoxicity for 3T3 and HeLa cells were depicted in figure 2 (A) and (B). Surprisingly, the cytotoxicity of the micrometer long nanowires exhibits little dependence on their aspect ratios for both cell lines. However, in a separated experiment, the cytotoxicity of 250 nm spherical gold nanoparticles (BBInternational) modified with mercapto acid was tested. The viability for both cell lines was measured to be larger than 80% with nanoparticle concentrations up to 10^7 nanoparticle/ml as shown in figure 3(A) and (B). Considering that the mass concentration of 10^7 nanoparticle/ml 250 nm spherical gold nanoparticles is equivalent to that of 10 μ m long nanowires with 200 nm diameter at a concentration of 1.6×10^5 nanowire/ml, the nanowires are more toxic than the spherical nanoparticles, which is probably due to the geometry of the nanowires. However, the exact origin of the cytotoxicity of the nanowires requires further investigation.

To further understand the internalization process of the nanowires, we have studied the uptake kinetics of aminothiols modified gold nanowires for both cell lines. The internalized nanowires were obtained by lysing the cells with alcohol and counted by a hemacytometer. The results for the nanowires with various aspect ratios are plotted in figure 4(A) and (B). The uptake kinetics for all sizes of nanowires was very similar in both HeLa and 3T3 cells. Both types of cells exhibited maximum uptake after 8 hours of incubation with the nanowires and decreased after reaching maxima. The maximum internalization efficiency was higher than 50% for both cell lines. The reason for the decrease in the uptake was due to the cell death at high concentration of nanowires as indicated in the viability test (figure 1). Another trend observed in this experiment was that the uptake efficiency for the longer

nanowire was lower than the shorter one except the 0.58 μm long gold nanowires, which were too short to determine the number of nanowires accurately. This trend agreed with the previous measurement using smaller nanorods (Bauer et al., 2003). Since nanowires with different aspect ratios exhibited the same cytotoxicity and the uptake efficiency of the nanowires decreased as the aspect ratio increased. We concluded that the internalized nanowires with higher aspect ratio were more toxic to both cell lines. This conclusion agreed with the conclusion that the gold nanowires were more toxic than the spherical (250 nm) gold nanoparticles and also explained why the LD_{50} of the nanowires was lower than the nanorods with smaller aspect ratio.

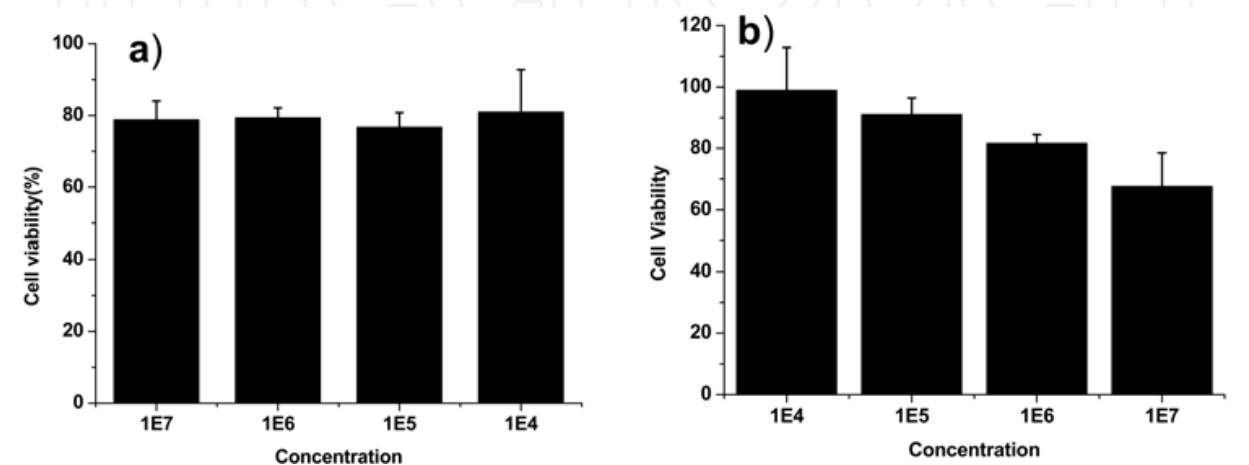


Fig. 3. The cytotoxicity of 250 nm gold nonparticles at different concentrations for (a) fibroblast cells (b) HeLa cell.

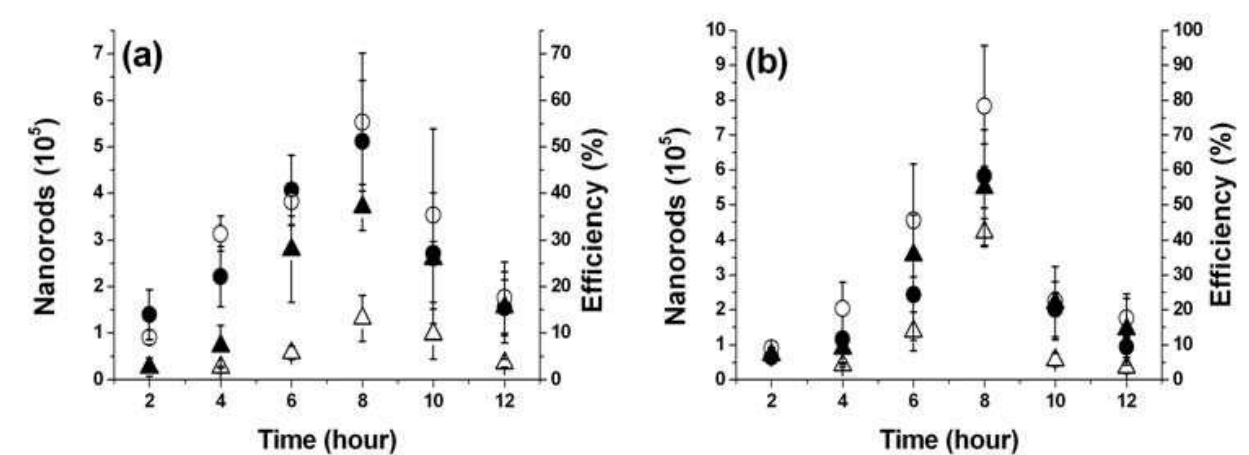


Fig. 4. The uptake of aminothiols modified gold nanowires with four different lengths in (a) fibroblast cell and (b) HeLa cell. 0.5 μm (solid circles), 1.8 μm (open circles), 4.8 μm (solid triangles), 8.6 μm (open triangles).

The cytotoxicity of silver nanowires was investigated by using 5 μm long silver nanowires with four different coatings: positive charge (amino group), negative charge (carboxyl group), hydrophobic alkyl group and bovine serum albumin (BSA) coating. After 24 hour incubation, we found that the cytotoxicity of silver nanowires increased as the number of silver nanowires and the cytotoxicity behavior was very similar to gold nanowires for both cell lines as shown in figure 5(a) and (b).

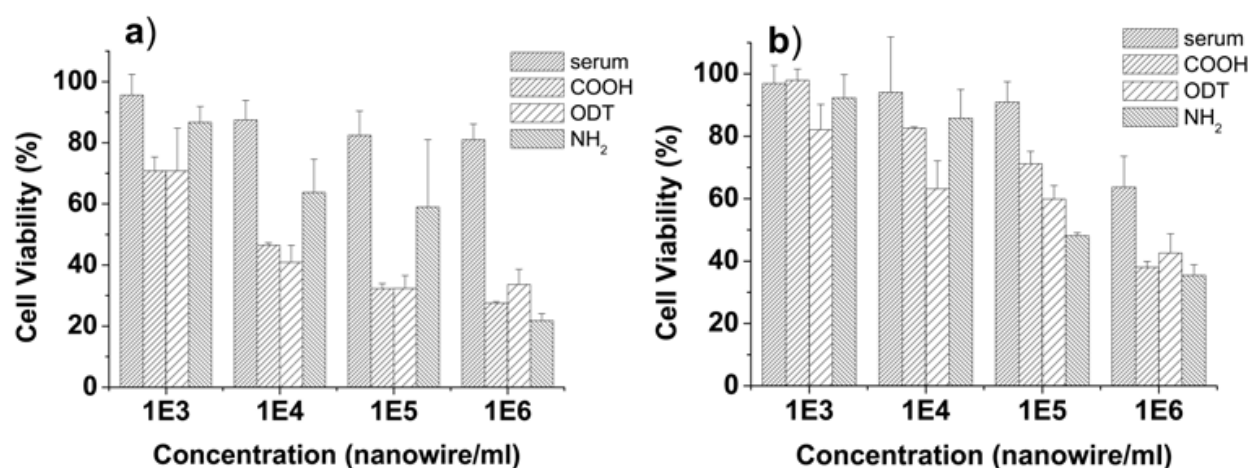


Fig. 5. Cytotoxicity for a) fibroblast and b) HeLa cells in the presence of various surface functionalized silver nanowires.

5. Nanowires as living cell probes

To use nanoparticles for living cell studies, these nanomaterials must remain stable in the intracellular environment and do not disturb normal cellular biochemical activities. Several types of nanomaterials such as magnetic, polymeric, metallic, semiconductor nanoparticles or nanowires have been introduced for the cellular study. For example, the capability of gold nanoparticles to incorporate secondary tags such as peptides to target specific cell types has been investigated (Goodman et al., 2004). In this study, the gold nanoparticles have been functionalized with cationic and anionic side chains. The carboxylated modification on the gold nanoparticles was found to be nontoxic to the cells. In contrast, the cationic side chain bound to the gold nanoparticles exhibited moderately toxicity. It was found that the toxicity of gold nanoparticles was related to their interactions with the cell membranes. In another study, the intracellular delivery of quantum dots (QDs) for live cell labeling and organelle tracking (Defus et al., 2004) were demonstrated. The QDs modified with polyethylene glycol (PEG) were mixed with different transfection reagents and then were delivered to the interior of the HeLa cells. The flow cytometry was used to quantify the amount of QDs delivered to the cells. It was found that QDs often tended to accumulate in vesicles and distributed non-homogeneously in the cytoplasm (Chen & Gerion, 2004).

As regarding to metallic nanoparticles, recent studies have shown that it was possible to use metallic nanoparticles for the targeted nuclear delivery. The nuclear targeting has been achieved by multifunctional gold nanoparticle-peptide complexes (Tkachenko et al., 2003). 20 nm diameter gold nanoparticles were modified with bovine serum albumin (BSA) bound with various cellular targeting peptides. To enter the nucleus of HepG2 cells, these nanoparticles must carry both receptor-mediated endocytosis (REM) and nuclear localization signal (NLS) peptides. Non-spherical nanorods have also been demonstrated capable of conducting gene delivery. It was shown that the conjugation of DNA plasmid and targeting ligands can be achieved simultaneously in a spatially defined manner. For example, it was demonstrated that gene delivery can be achieved using bifunctional Au/Ni nanorods (Salem et al., 2003). In this approach, the carboxylate terminal group was first attached to the Ni segment. Subsequently, the plasmids were bound to the protonated amines on the surface of nickel segment through electrostatic interactions. The transfferrin

was bound to the gold segment of the nanorods through thiolate linkage. These dual-functionalized Au/Ni nanorods were used in a *vitro* transfection experiment using human embryonic Kindney (HEK 293) mammalian cell line.

We have introduced functionalized gold nanowires as new probes (Kuo et al., 2007). Using various types of functionalization, it was possible to probe the local environment inside the cells by confocal microscopy. To observe the internalization process of nanowires, 5 μm long gold nanowires (200 nm in diameter) coated with serum were added into a glass bottom culture dish, which were placed in a CO_2 incubator on an inverted microscope. The images were recorded by a CCD camera every 10 minutes as shown in figure 6. The internalization process of the gold nanowire to the HeLa cells was recorded by the time lapse microscopy for 4 hours. We found that the micrometer long metallic nanowires can be internalized by cells without damaging the cells, which is important in cellular study.

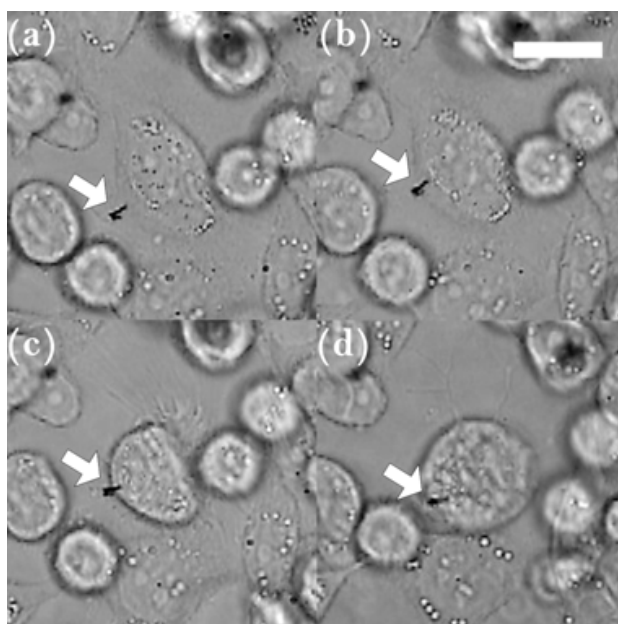


Fig. 6. The phase contrast image of the serum coated gold nanowires internalized by HeLa cell. Bar: 20 μm .

To investigate the capability of delivering DNA molecules into the cells through the nanowires, the 5 μm long gold nanowires were functionalized with aminothiols, which covered the nanowire surfaces with positive charges. The negatively charged plasmid DNA molecules were attached to the nanowires through electrostatic interaction. To demonstrate that the surface functionalized nanowires can carry the plasmids into the cytoplasm, a green fluorescence protein expressing plasmid (pAcGFP1-Actin, BD) was coated on the gold nanowires. For visualization purposes, the plasmid DNA on the nanowire surfaces were further labeled with YOYO-1, which emitted a strong green fluorescence when bound to a double strain DNA, and the cells were stained with Image iT LIVE Plasma Membrane and Nuclear Labeling Kit as shown in figure 7. From the confocal image, it can be clearly seen that the 5 μm long functionalized gold nanowires coated with plasmid were located inside the cell. Knowing that the gold nanowires can delivery plasmid into cells, it is important to investigate the functionality of plasmids on the gold nanowires. Shown in figure 8 is the combined DIC and confocal image of the fibroblast cell expressing GFP using gold nanowires as the gene carrier. This experiment confirmed that the micrometer long

aminothiol modified gold nanowires can not only protect plasmid DNA molecules from degradation but also release plasmid NDA molecules inside the cells.

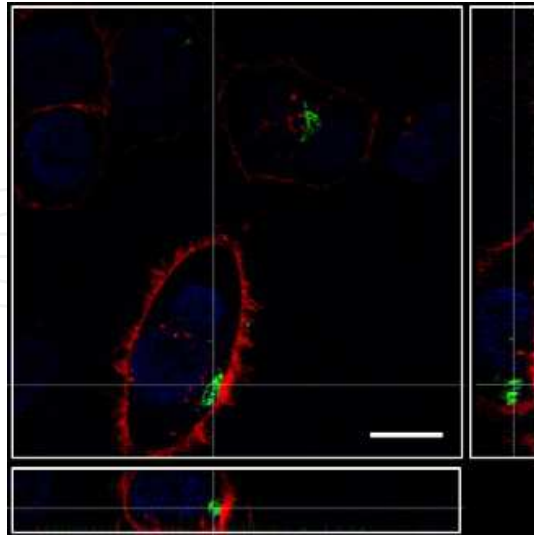


Fig. 7. Stacked confocal image of nanowires in HeLa cell

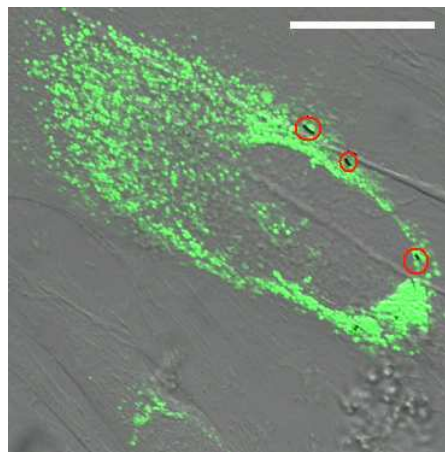


Fig. 8. The combined DIC and fluorescence image of plasmid coated nanowires and fibroblast cell expressing green fluorescence proteins. Nanowires are indicated by the red circles. The bar is 20 μm .

To further demonstrate that it was possible to monitor the local environment of the nanowires, the LysoSensor Yellow/Blue, which exhibited pH dependent dual emission spectra for intracellular environment study, was modified to the surface of nanowires. The LysoSensor coated gold nanowires inside the 3T3 cells were measured by confocal microscope as shown in figure 9. In the acidic environment, the emission of the LysoSensor dyes is in the range of 500 to 600 nm, which shifts to 410 to 500 nm region in the less acidic environment. In this experiment, the LysoSensor Yellow/Blue dyes was incubated with the 2 μm long amino-modified gold nanowires. LysoSensor coated nanowires can be used to probe to the acidic organelles such as endosomes or lysosomes. It was found that the emission from LysoSensor coated nanowires was in the green region indicating the environment around nanowires was less acidic. By monitoring both the DIC image of the nanowires and confocal image of the probe molecules as a function of time, the evolution of

the local environment around the nanowires can be explored. In general, this approach can be extended to other types of probe molecules or biological assays.

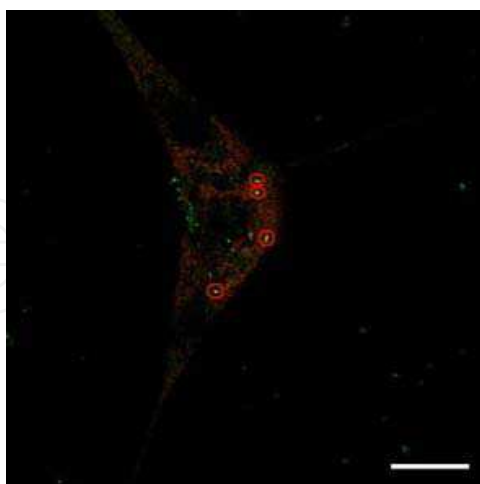


Fig. 9. The confocal image of the gold nanowires coated with LysoSensor inside HeLa cell. Bar: 20 μm .

6. Nanowire for gene delivery

Gene therapy is one of the promising strategies in the treatment of various diseases such as AIDS, cancer. The challenge of *in vivo* gene therapy is to develop safe and efficient gene delivery system. For the gene therapy to be used in the clinical applications, it requires the development of efficient DNA delivery vehicles that can be synthesized both easily and in large quantities. It has been demonstrated that the viral vectors can provide the most efficient gene transfer. Viral vectors were originally developed as an alternative to the transfection of the naked DNA in the molecular genetic experiments. So far, several types of viral vectors have been developed for the gene transfection including retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses. However, the immuno-response induced by the viral vectors has raised a lot of concerns of using viral vector as the gene delivery system.

To reduce the immuno response, non-viral vectors, such as calcium phosphate, cationic lipids, cationic polymer, and dendrimers, have been recently developed. These non-viral vectors have been shown to exhibit lower transfection efficiency than the viral vectors. However, they are believed to be safer than viral vectors. Recent research efforts for the development of non-viral vectors have been focused on the improvement of their transfection efficiency. Non-viral vector approach usually involved the use of plasmid DNA or oligonucleotides, which are considered to be safer than the viral vectors. With recent advances in nanotechnology, researchers are now routinely synthesizing various nanomaterials with desired properties. Therefore, nanomaterials may also be used as the carriers for gene delivery. In recent reports, polymer nanoparticles or nanowires have been introduced as the vehicles for the drug delivery to enhance the cellular uptake efficiency. For this purpose, nanomaterials have been developed to incorporate with DNA molecules such that they can directly facilitate cellular interactions. The nanomaterial based drug delivery systems are being developed to overcome the barrier of size, stability, charge density, and biodistribution, which may lead to novel therapeutic strategies for the treatment of a variety of inherited or acquired diseases.

Since the metallic nanowires are capable of carrying DNA into cells, they may be used as the vectors for gene delivery. To measure the optimal condition binding efficiency of the plasmid to the nanowires, we have examined 5 different concentrations of 5 μm long aminothiols modified gold nanowires (5×10^3 , 2.5×10^4 , 5×10^4 , 2.5×10^5 , 5×10^5 / ml), which were mixed with 50 ng of the DsRed-Monomer-golgi plasmid DNA for 24 hours (Kuo et al., 2008). The complexes with nanowires were then mixed with XYBR dyes and loaded in an agarose gel. The result is shown in figure 10 where the plasmid bands in lane 5 and 6 were missing that at these concentrations all the plasmids were bound to the nanowires. Therefore, we estimated that the binding efficacy for the plasmid DNA molecules on the 5 μm long aminothiols modified nanowires were about 1 pg/nanowire. The zeta potential of the plasmid bonded gold nanowires was measured to be $-9.3 \pm 0.5 \text{ mV}$, which clearly indicated that DNA bound to gold nanowires to form complex. The DNA gold nanowire complex could protect the DNA molecules from the attack of the DNA nucleases.

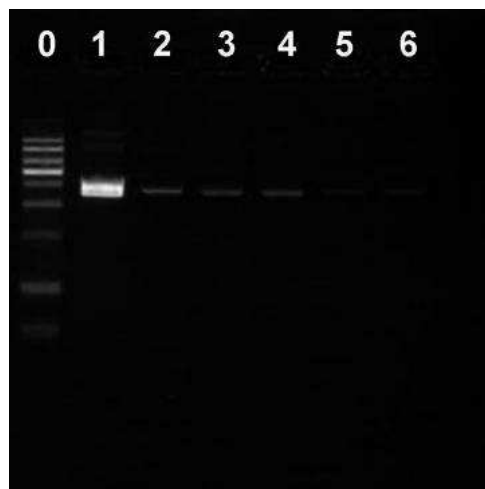


Fig. 10. Agarose gel electrophoresis of the aminothiols modified gold nanowires and plasmid. Lane 0 is the DNA marker. Lane 1 is the 50 ng plasmid DNA without nanowires. Lane 2 to 6 are the mixture of 50 ng plasmid DNA with increasing numbers of the gold nanowires (5×10^3 , 2.5×10^4 , 5×10^4 , 2.5×10^5 , 5×10^5).

To test the transfection efficiency of the aminothiols modified gold nanowires, 5 μm long nanowires with concentration of 10^5 nanowire/ml was mixed with the 50 ng of DsRed-Monomer-golgi vector and incubated over night. Two cell lines, NIH 3T3 and HeLa S3, were used in this experiment. The control experiments were conducted with dendrimer based commercial transfection agent (PolyFect) and calcium phosphate. The transfection efficiency for the naked plasmids was also measured. The viability of the cells incubated with the transfection reagents was checked separately by staining the cell with trypan blue 24 hours after the transfections. The transfection efficiency was calculated by measuring the ratio of the number of cell exhibited red fluorescence to the total number of the cells on the surfaces and normalized to the cell viability. Shown in figure 11 are the combined DIC and fluorescence images of 3T3 cells 24 hours after transfection using four different transfection reagents. The viability of the 3T3 cells 24 hours after transfection was measured to be 90%, 65%, 65% and 71% for gold nanowires, PolyFect, calcium phosphate and naked DNA, respectively. It is clear that aminothiols modified gold nanowires exhibited the highest transfection efficiency with very low toxicity for 3T3 cells. Previously, we have shown that

more than 30% of the gold nanowires could be internalized by the 3T3 cells within 8 hours, which may explain the high transfection efficiency of the micrometer long gold nanowires.

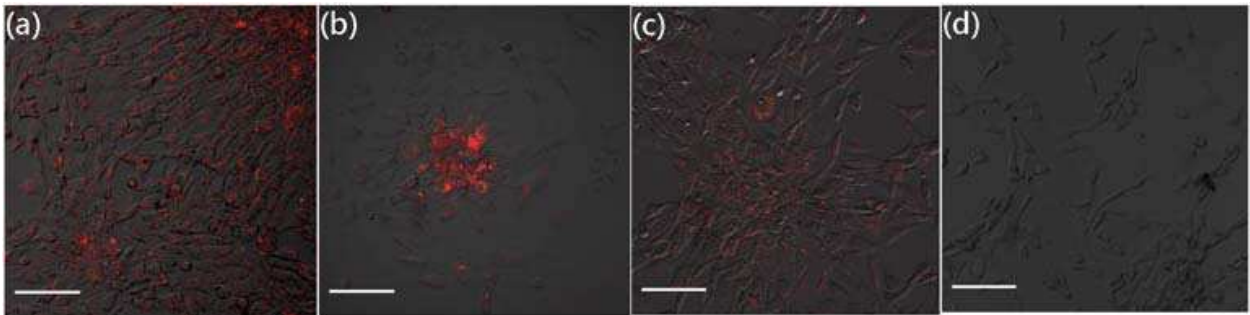


Fig. 11. The combined DIC and confocal images of 3T3 cells 24 hours after using (a) gold nanowires (b) PolyFect (c) calcium phosphate (d) naked DNA as the transfection reagents. Bar: 100 μ m

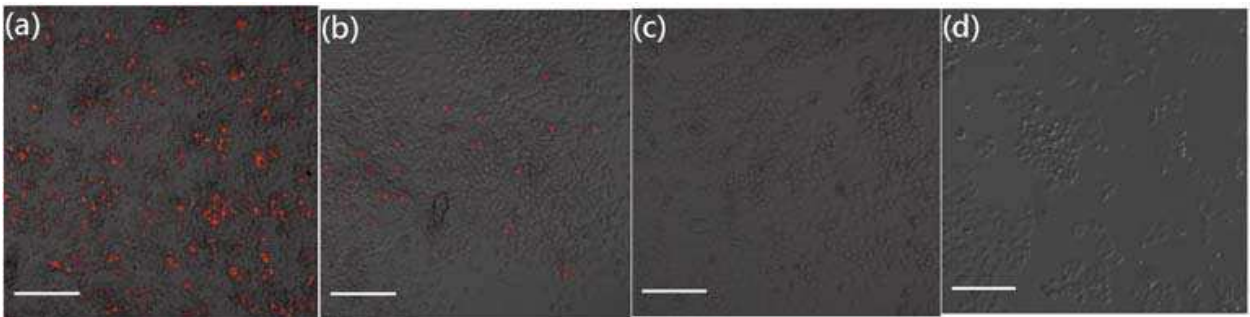


Fig. 12. The combined DIC and confocal images of HeLa cells 6 days after using (a) gold nanowires (b) PolyFect (c) calcium phosphate (d) naked DNA as the transfection reagents. Bar: 100 μ m

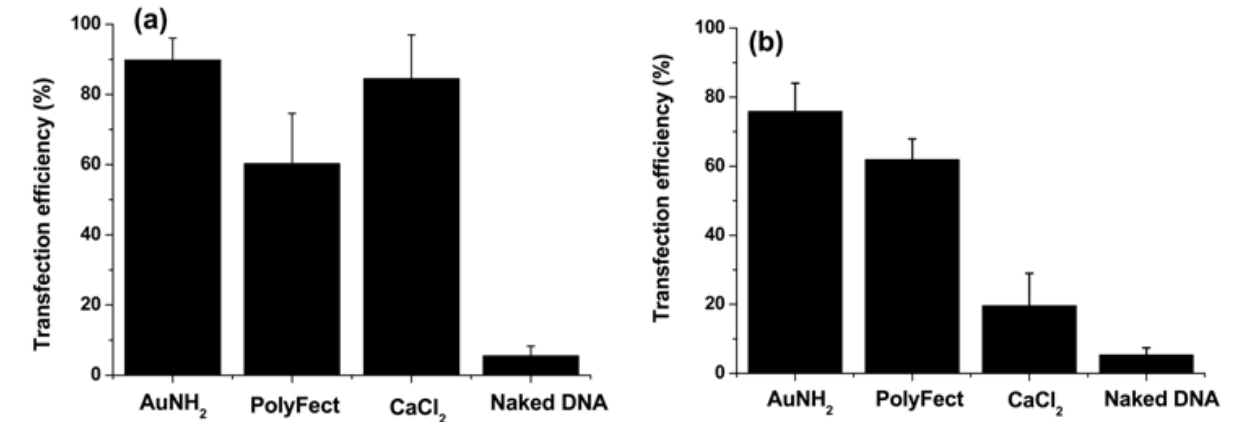


Fig. 13. The transfection efficiency measured for different transfection reagents for (a) 3T3 cells (b) HeLa cells.

As for the HeLa cells, the condition changed. Very little amount of cells were transfected 24 hours after incubation with all transfection reagents despite of the fact that more DNA molecules were used. After a few days, the cells started to exhibited red fluorescence. Shown in figure 12 are the combined DIC and fluorescence images of the transfected HeLa cells six days after transfection. The viability of the HeLa cells 24 hours after incubation with

the transfection reagents was measured to be 95%, 87%, 86% and 90% for gold nanowires, PolyFect, calcium phosphate and naked DNA, respectively. HeLa cells seemed to resist to the addition of the transfection reagents. Therefore, lower cytotoxicity as well as lower transfection efficiency was measured for the HeLa cell lines. The transfection efficiencies of four different reagents for both cell lines are summarized in figure 13. In both cases, the gold nanowires exhibited the highest transfection efficiency while very little cytotoxicity to both cells lines was measured. To achieve higher transfection efficiency for other transfection reagents, DNA loading in the μg region was needed (Akita et al., 2004). Therefore, we concluded that the micrometer long nanowires could effectively deliver plasmid DNA into both 3T3 and HeLa cells.

7. Conclusions

Metallic nanowires with various aspect ratios have been synthesized using AAO template deposition. Four different surface modification schemes including serum, alkanethiols, mercapto acids and aminoalkylthiols were used to functionalize the surfaces of the metallic nanowires. It was found that all surface modifications except aminothiols produced negatively charged gold nanowires. Therefore, the amino presenting gold nanowires were used bind the negatively charged plasmid DNA molecules.

The cytotoxicity of the micrometer long metallic nanowires with various aspect ratios and surface modifications have been investigated. It was found that the serum coated nanowires exhibited the least cytotoxicity with a LD_{50} value around $150 \mu\text{g}/\text{ml}$, which was less than those measured for the smaller gold nanoparticles. All other surface functionalized nanowires possessed some degree of toxicity, which depended on the surface charge. Among them, the mercapto acid modified nanowires were the most toxic nanowires. For the same type of surface modification, HeLa cell, which can grow in suspension, were found to be more resistant to the addition of the nanowire solution. As for the nanowires with different aspect ratio, the cytotoxicity experiments indicated that nanowires with different aspect ratios exhibited the same degree of toxicity. However, the uptake efficiency for the shorter nanowires was measured to be higher than the longer nanowires. Therefore, we concluded that the internalized nanowires with higher aspect ratio were more toxic than the shorter one, which explained that the LD_{50} value for the nanowires was lower than that of the low aspect ratio nanorods. This conclusion also agreed with the cytotoxicity experiment for the spherical nanoparticles where the nanowires were found to be more toxic than the spherical nanoparticles.

We have also demonstrated that the $5 \mu\text{m}$ long nanowires with surface modifications were capable of delivering plasmid DNA molecules, which has been visualized and recorded on an optical microscope. In addition, LysoSensor was attached to the nanowires revealing the local environment of the nanowires. By monitoring the color change of the Lysosensor on the nanowires, it was found the nanowires stayed in the less acidic environment indicating that nanowires never escaped from the endosome/lysosome complexes.

The transfection efficiency of the surface functionalized nanowires has also been studied. It was found that the transfection efficiency of the aminethiol modified gold nanowires was the highest among the tested transfection reagents while almost no cytotoxicity was observed for gold nanowires under our experimental condition. It was also shown that it was possible to trace the nanowires inside the cells with sub-micrometer resolution. Therefore, we concluded that the micrometer long nanowires could be used for probing

living cells with several advantages including easy fabrication and surface modification process, high transfection efficiency with very low cytotoxicity and readily observable by a microscope.

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Electrodeposited Nanowires and their Applications

Edited by Nicoleta Lupu

ISBN 978-953-7619-88-6

Hard cover, 228 pages

Publisher InTech

Published online 01, February, 2010

Published in print edition February, 2010

The book offers a new and complex perspective on the fabrication and use of electrodeposited nanowires for the design of efficient and competitive applications. While not pretending to be comprehensive, the book is addressing not only to researchers specialized in this field, but also to Ph.D. students, postdocs and experienced technical professionals.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Chiung-Wen Kuo and Peilin Chen (2010). The Applications of Metallic Nanowires for Live Cell Studies, Electrodeposited Nanowires and their Applications, Nicoleta Lupu (Ed.), ISBN: 978-953-7619-88-6, InTech, Available from: <http://www.intechopen.com/books/electrodeposited-nanowires-and-their-applications/the-applications-of-metallic-nanowires-for-live-cell-studies>

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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