

# Toxicity of Nanomaterials to Living Cells

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## ABSTRACT

We have studied the toxicity of luminescent silica nanoparticles to living cells from the whole cell level to the molecular level, such as the effects of the nanoparticles on DNA/protein damage, DNA repair activity, cell apoptosis, cell proliferation and death. Several biological approaches were employed to characterize the toxicity of the nanoparticles, including Vybrant apoptosis assay, MTT assay, DNA repair enzyme activity assay and comet assay. Preliminary results showed that the nanoparticles have no apparent effects on survival in several types of living cells up to 72 hours of incubation. Furthermore, luminescent silica nanoparticles do not cause significant DNA damage (strand breaks) compared to controls.

**Keywords:** nanoparticles, luminescence, toxicity, cell apoptosis, DNA damage

## 1 INTRODUCTION

Nanotechnology in combination with biomedical and other biotechnological developments promises to produce major breakthroughs and revolutionary tools in biomedical analysis [1]. One of the most promising applications of such combinations is to apply luminescent nanomaterials as biomarkers to identify target cells from a matrix. Due to the excellent signaling capabilities of luminescent nanomaterials, trace amounts of target cells can be recognized down to a single one without pre-enrichment of the targets [2]. This has demonstrated a great potential of luminescent nanomaterials for early cancer diagnosis *in vitro* based on sensitive measurement of protein changes in the cancer cells. However, the major concern, whether these luminescent nanomaterials would cause toxic effects to living systems, has not been addressed.

So far, several important luminescent nanomaterials, including quantum dots (QDs) [3-5] gold and silver nanoparticles [6,7] and luminescent silica nanoparticles [8], have been developed. These nanomaterials exhibited high signal enhancement in the detection of trace amounts of biomolecules. Among them, newly developed luminescent silica nanoparticles (NPs) have demonstrated great promise as luminescent biomarkers for trace amounts of biological sample analysis [2,9]. To further drive the application of

luminescent silica nanoparticles to the biomedical field and eventually explore the potential for cancer diagnosis and therapy, the investigation of NPs toxicity to living system is critically needed.

To meet this need, we investigated the toxicity of luminescent silica NPs to living cells. Based on recent initial efforts on toxicological characterization of other types of nanomaterials, the toxicity of nanomaterials varied by types, organisms, and matrix. For example, carbon nanotubes [10] inhibited cell growth by inducing cell apoptosis and decreasing cellular adhesion ability. Cationic modified metal nanoparticles were moderately toxic; whereas anionic modified metal nanoparticles were nontoxic[11]. By employing different biological methods, our approach was designed to investigate the effects of luminescent silica NPs to living cells from the whole cell level to the molecular level. The effects of nanoparticles on protein/DNA damage, DNA repair activity, cell necrosis and apoptosis, cell proliferation and death were studied in detail. We expected that this study provides critical information for biotechnological and biomedical applications of luminescent silica nanoparticles.

## 2 METHODS

The toxicological characterization of luminescent silica NPs to living cells was carried out in two aspects. (1) At the whole cell level. These experiments investigated the effects of luminescent silica NPs on cell proliferation, death, necrosis and apoptosis to determine whether the toxic effect presents macroscopically. The methods used in this experiment were Vybrant apoptosis assay and MTT assay. (2) At the molecular level. The experiments microscopically investigated the toxic effect (strand breaks) of nanoparticles to living cells using the DNA repair activity assay and comet assay.

## 3 RESULTS AND DISCUSSION

### 3.1 Analysis of Cell Apoptosis and Necrosis Using Vybrant Apoptosis Assay

Vybrant apoptosis assay was carried out to investigate the effect of nanoparticles on cell apoptosis. During Vybrant apoptosis assay, two kinds of dye molecules were added to the cell solution. The size of green dye (YO-PRO-1) was

smaller than the red one (Propidium Iodide: PI). As apoptosis occurred, the cell membrane was slightly permeant and thus allowed small YO-PRO-1 molecules to penetrate the cells. Therefore, the green color showed cell necrosis on the images of Vybrant apoptosis assay results. However, as necrosis occurred, the wide channels appeared on the cell membrane and bigger red dye molecules (PI) entered the cell. Consequently, the red color was evidence for cell necrosis on fluorescent images.

We investigated the effect of different concentrations of nanoparticles on living cells using Vybrant apoptosis assay. A few cells showed apoptosis phenomenon (less than 20%) (Figure is not shown) which were similar to the control sample. Although the nanoparticle concentration reached was as high as  $10^{11}$  NPs/mL, the toxic effect was minimal. The cells did not appear to proliferate as in the normal conditions. To reach the limit of the nanoparticles concentration at which cell proliferation will be inhibited by nanoparticles, we tested an unreasonable NP concentration ( $5 \times 10^{11}$  NPs/mL). As expected, the majority of cells died. We assumed that a crowd of NPs blocked cell growth at extremely high concentration of NPs. To confirm this assumption, we further carried out an MTT Assay.

### 3.2 Investigation of Cell Proliferation and Apoptosis by MTT Assay

To further verify cell apoptosis results, we detected the cell apoptosis phenomenon from a different approach via MTT assay. Tetrazolium in a dye molecule can be converted

into a formazan product whose absorbance at 570 nm is directly proportional to the amounts of living cells when the dye is added to the living cells. Thus, MTT assay is conducted by adding the tetrazolium dye solution to cell culture media. After converting, the visible absorbance of the formazan reflects the amounts of surviving cells in samples. This method provides another tool to study cell proliferation and further toxicity of NPs to cells.

The MTT assay showed that although the approach was different with Vybrant apoptosis assay, the two results were consistent. With concentrations of NPs lower than  $1 \times 10^{10}$  NPs/mL, the NPs showed no apparent damage to living cells. At the concentrations of NPs exceeded  $1 \times 10^{10}$  NPs/mL, some toxic effects were observed (Figure 1).

### 3.3 Effect of Luminescent Silica NPs on Protein Signaling Via Activity Assay

MTT assay and Vybrant apoptosis assays provided information on effects of luminescent silica nanoparticles to living cells from the level of whole cells and focused on cell proliferation and apoptosis. Although the results showed that nanoparticles have no apparent toxic effects on cell proliferation, additional evidence was sought to demonstrate that the nanoparticles were safe to living cells. Therefore, further toxicological characterization of nanoparticles to living cells needs to be performed at the molecular level inside cells.

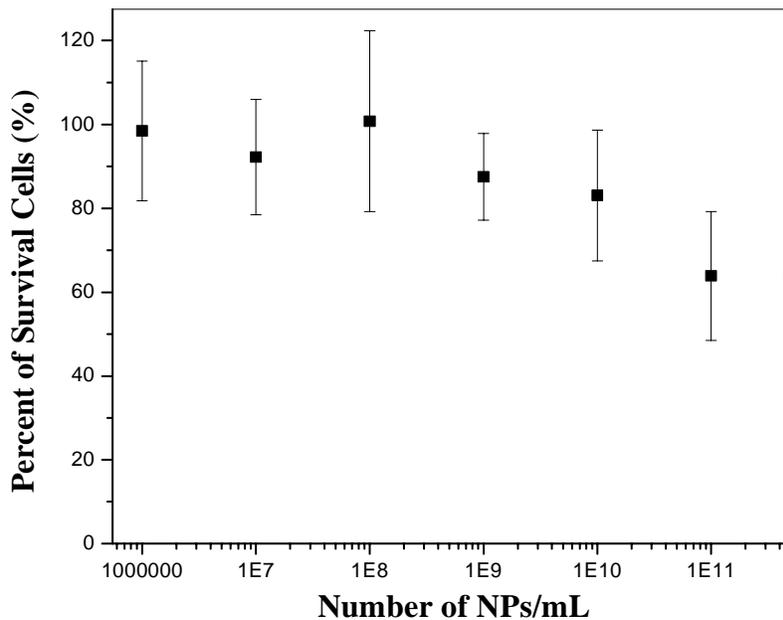


Figure 1: Absorbance of Formazan in the MTT Assay.

A549 cells incubated with different concentrations of NPs for 48 h. Absorbance wavelength was at 570 nm.

Table 1: Ratio of the cleaved to uncleaved bands of NPs incubated with A549 cells based on DNA repair protein activity assay (tail length units: cm)

Incubate time	Negative control	NPs:Cells (1000:1)	NPs:Cells (5000:1)
48 hours	0.088±0.024	0.135±0.005	0.108±0.031
72 hours	0.077±0.023	0.071±0.009	0.064±0.001

We studied the DNA repair protein molecule changes inside the cells after growing cells with nanoparticles. In the electrophoresis autoradiograph of protein extracts from the cell lysates, the amounts of proteins were represented by the intensity and the area of the bands. In this experiment, the first band represented the total uncleaved substrates (26 bp). The second band represented the cleaved substrates (9 bp). The rationale is that increased DNA repair activity reflects stronger DNA damage as responses. When DNA damages, the DNA repair activity is increased to cleave the substrate resulting two bands. Figures (does not show) clearly showed that there was no difference between protein molecules incubated with nanoparticles and proteins from control cells. To further verify the result, we extended the time of nanoparticles incubating with cells up to 72 hours. Similar results were obtained. Furthermore, the ratios of the cleaved to uncleaved in each sample were calculated and compared with the negative control (Table 1). The data clearly showed that the ratios of nanoparticles treated cells were very similar to those of negative controls. These preliminary results demonstrated that NPs have no visible toxic effects on DNA repair proteins.

### 3.4 Effect of Luminescent Silica NPs on DNA Damage Based on Comet Assay

The comet assay is also called single cell electrophoresis assay. This assay can reveal whether nanoparticles cause DNA damage by counting the “comet” tail length. The longer comet tails could be observed from severe DNA damaged cells. Different amounts of luminescent silica nanoparticles were added to cell culture dishes during cell growth (1K and 5K represent the ratio of the number to cells that are 1000:1 and 5000:1 respectively. Negative control samples have only PBS containing medium). The fluorescent images of comet experiments on human lung cells (A549 cells) at different incubation times and with different amounts of nanoparticles were obtained using a confocal fluorescence microscope.

The average lengths of A549 cell comet tails were calculated. The comparisons of the tail lengths between negative control cells (without NPs during cell culture) and nanoparticles-incubated cells demonstrated that the tail lengths of negative controls and sample cells have no visible difference. The preliminary results imply that luminescent silica NPs have no obvious toxic effects on cells, even though the NPs amounts are a few thousand per cell.

## 4 CONCLUSIONS

In conclusion, our preliminary results have shown that luminescent silica nanoparticles have no apparent effects on survival in several types of living up to 72 hours based on Vybrant apoptosis assay, MTT assay, and comet assay. Moreover, the NPs demonstrated no considerable DNA damage (strand breaks) compared to controls based on comet assay. These data suggested that it may be promising to further explore the applications of luminescent silica nanomaterials to the biomedical field and eventually to apply the NPs to cancer diagnosis and therapy. We expect that this study could provide critical information for biotechnological and biomedical applications of luminescent silica nanomaterials.

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