The Intracellular Co- localizations of Different Size of Gold Nanoparticles

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ABSTRACT

Gold nanoparticles (GNPs) are widely used in biological and clinical applications due to their favorable optical and chemical properties. These properties make them ideal probes for biological and cell imaging applications. The scientific understanding of the intracellular uptake of GNPs is required for biomedical applications, such as drug delivery and therapeutic application. In this study, we conducted a series of studies using different sizes of gold nanoparticles, including 3 nm, 10 nm, 26 nm and 50 nm to determine the influence of size variations on the intracellular profiles of nanoparticles. We infected human body epithelial type 2 (HEp-2) cells with different size of gold nanoparticles and incubated for different time intervals (1, 2, 4, 12, and 24h) followed by imaging using different microscopic techniques, such as scanning electron microscopy (SEM), atomic force microscopy (AFM), and confocal microscopy. Our results showed that after 1 h incubation, 3, 10 and 26 nm GNPs entered into the nucleus, whereas 50 nm particles accumulated around nucleus. According to confocal images, single GNP emitted blue color, whereas highly aggregation leads to emission in red color range in the nucleus.

1. INTRODUCTION

In recent decades, metal nanoparticles have been extensively used in biological and biomedical applications (1, 2). Their nanoscale sizes make them similar to cellular components and proteins, hence, nanoparticles can easily bypass natural barriers (3). Metal nanoparticles exhibit remarkable optic, electronic and chemical properties, as their properties can be tailored by size variations (4). Recent studies showed that the size diversity of nanoparticles plays a significant role for their extracellular and intracellular mechanisms (1,5, 6). Although the cellular deposition of nanoparticles has promising potential for applications in diagnosis and sensing, but limited studies have been done to understand the cellular profile mechanisms of nanoparticles.In our study, we used four different sizes (3, 10, 26, and 50 nm) of GNPs in order to identify the influence of size variations on the intracellular profiles of nanoparticles, because recent few decades, gold nanoparticles have received promising potential in biological and clinical applications due to their excellent optical and chemical properties (7). In the first part of our study, we measured the toxicity of GNPs in HEp-2 cells using two different techniques, MTS assay and flow cytometry. In second step, we infected HEp-2 cells with different size of gold nanoparticles and incubated for different time intervals (1, 2, 4, 12, and 24h) followed by imaging using different microscopic techniques, such as scanning electron microscope (SEM), atomic force microscope (AFM), and confocal microscope. Our results showed that the most of GNPs accumulated into the nucleus.

2. MATERIALS & METHODS

2.1. Gold Nanoparticles

Four different sizes (3, 10, 26, and 50 nm) of gold nanoparticles (GNPs) were purchased from Nanoparts™ Inc. These GNPs were < 95% pure with a density of 0.041-0.065 g/cm^3.

2.2. Cells

HEp-2 cells were purchased from American Type Culture collection (ATCC, Manassas, VA; CCL-23) and were propagated by standard methods using Minimum Essential Medium (MEM) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-Glutamine, 75 U/ ml Penicillin, 100 µg/ml Kanamycin and 75 µg/ml Streptomycin, and cells were routinely grown at 37°C in a humidified 5 % CO_2 atmosphere.

2.3. MTS Cytotoxicity Assay

The cytotoxicity of GNPs was determined using the MTS dye reduction assay in HEp-2 cells. Cells were

seeded in a 96-well plate at a density of 2.0 x 10^4 cells/well and incubated overnight at 37°C. Then the cells were incubated in 100 µl 10% fetal bovine serum containing selected medium supplemented with (0.5, 1, 5, 10, 25, and 50 µg/ml) of GNPs. After 24 h, the medium was removed and the cells were rinsed twice with sterile PBS. Next, 10 ul of MTS (5 mg/ml) solution was added into each well and allowed to react for 4 h at 37°C. Endothelial Basal Medium EBM™-Phenol Red Free (150 µl) was added to each well and the plate was incubated for 30 min at room temperature. Absorbance at 490 nm was measured with an ELISA plate reader (TECAN US Inc., Durham, North Carolina).

2.4. Confocal Microscopy Analysis

After plating the HEp-2 cells in an 8-chamber slide and incubation for 24 h, desired concentrations of gold nanoparticles were added to each chamber and incubated for different time intervals (1h, 2h, 4h, 12h, and 24h). This was followed by staining nucleus with Hoechst 33342, trihydrochloride, trihydrate - FluoroPure™ according to manufacture's instruction.

2.5. Scaning Electron Miroscopy Analysis

HEp-2 cells were grown in cover slips in a 12-chamber slide and incubated for 48 h. Desired concentrations of gold nanoparticles were added to each chamber and incubated for different time intervals (1, 2, 4, 12, and 24 h). This was followed by fixing cells with 200 µl methanol for 15 minutes, 200 µl distilled water for 15 minutes, and washed with 70%, 90%, and absolute alcohol for 5 minutes each. SEM images were taken using Carl Zeiss CrossBeam® Workstation AURIGA® in Auburn University, Alabama.

2.6. Atomic Force Microscopy Analysis

AFM samples were prepared using the same method discussed above for SEM. AFM pictures were obtained using the NANOSCOPER2 AFM (Pacific Nanotechnology, Santa Clara, CA, USA). HEp-2 cells exposure to GNPs were placed onto a slide, dried and visualized under the microscope. Close contact mode and standard silicon cantilevers (Pacific Nanotechnology, Santa Clara, CA, USA) 450 µm in length and 20 µm in width were employed for imaging. The cantilever oscillation frequency was tuned to the resonance frequency of approximately 256 kHz. Both height and phase information were recorded at a scan rate of 0.5 Hz, and in 512 x 512 pixel format.

2. RESULTS & DISCUSSION

3.1. MTS Assay

Concentrations ranging from 0.5 to 50 µg /ml of each nanoparticle sample were tested for toxicity to HEp-2 cells by MTT assay (Fig. 1). Although GNPs have been reported as nontoxic our results showed that after 24 incubation, GNPs displayed significant toxicity, especially, 3nm particles reduced the cell viability approximately % 20at 50 µg/ml.

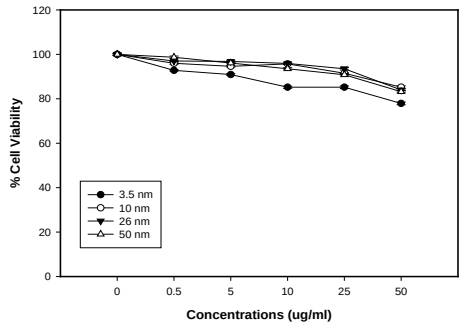

Figure. 1: HEp-2 cells were incubated for 24 h with various concentrations (0.5-50 µg/ml) of different size (3, 10, 26, 50 nm) GNPs.

3.2. Microscopy Studies

We started our microscopy studies imaging only HEp-2 cell without any GNPs as a control. Then, we imaged HEp-2 cells treated with different size of GNPs at given time intervals. For confocal studies, we used Hoescht 3332 (blue) to stain cell nucleus.

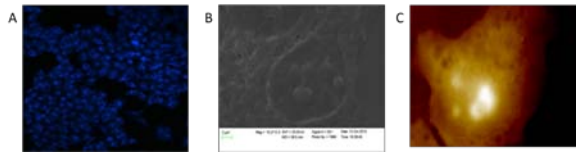

Figure 2. Control images; A) Confocal B) SEM C) AFM imaging of HEp-2 cells without any GNPs.

3.2.1. Confocal microscopy

Our confocal microscope study showed that single GNPs emitted blue color, whereas aggregation leads to emission in red color range. Our data confirmed that small nanoparticles emit energy with small wavelength (λ), whereas particles with larger surfaces release energy with larger wavelength. As GNPs enter cells, they accumulated in nucleus, resulting in emission of energy with larger wavelength. Thus, we observed aggregated particles with red color

fluorescence in the nucleus. However, after 1 incubation we observed less red color fluorescence for 50 nm particles indicating it's less accumulation rate into nucleus than other smaller size particles.

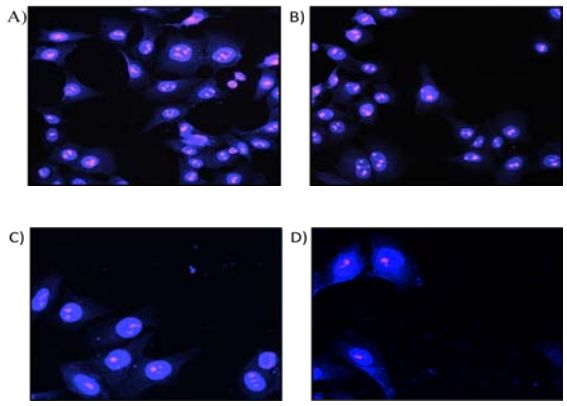

Figure 3. Confocal microscopy imaging of A) 3 nm, B) 10 nm, C) 26 nm, and D) 50 nm GNPs into HEp-2 cells after 1 h incubation.

Although 50 nm GNPs were aborted by nuclear pore complexes in 1 h interval, aggregated 50 nm GNPs also were visible at same red fluorescence range into the nucleus at following time intervals. Our data showed that even after 24 h incubation, all sizes of GNPs kept localizing into the cell nucleus.

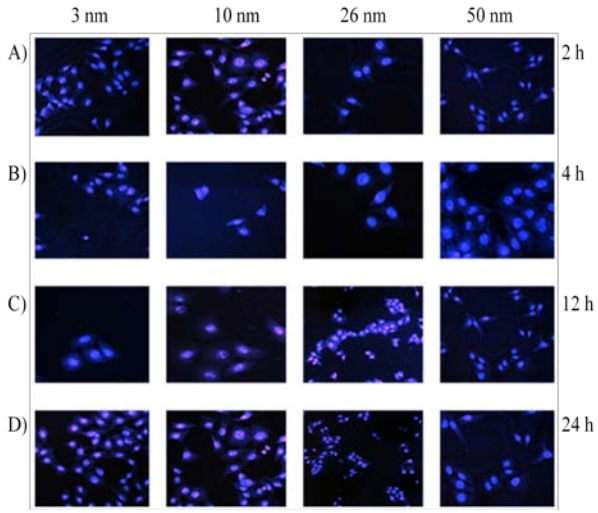

Figure 4. Confocal microscopy imaging of 3, 10, 26, and 50 nm gold nanoparticles into HEp-2 cells after, a) 2 h, b) 4 h, c) 12 h, d) 24 h incubations**.**

3.2.2. Scanning Electron Microscopy Study

Our SEM results showed that the most of GNPs have entered cell nucleus at different rates depending on the particle sizes. After 1 h incubation, 3, 10 and 26 nm gold nanoparticles entered to nucleus, whereas 50 nm particles highly accumulated around nucleus.

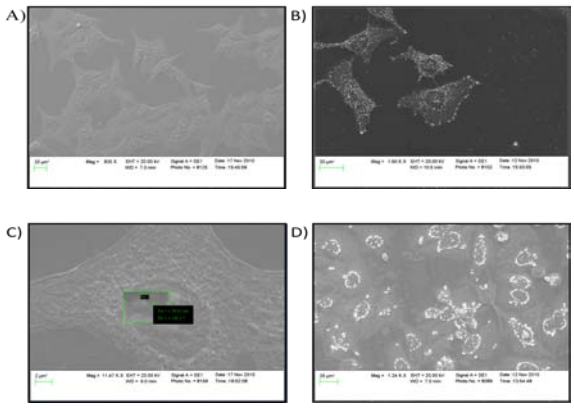

Figure 5. SEM imaging of A) 3 nm, B) 10 nm, C) 26 nm, and D) 50 nm GNPs into HEp-2 cells after 1 h incubation.

Our SEM results corresponded with our confocal microscopy study that most of the GNPs aggregated into nucleus. We continued to observe high amount of GNPs into nucleus. We were not able to detect single particles with SEM microscopy, but could determine highly accumulated site in the nucleus.

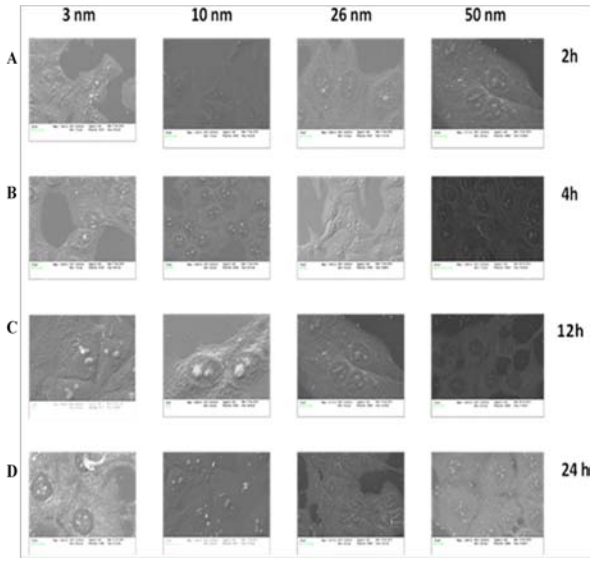

Figure 5. SEM imaging of 3, 10, 26, and 50 nm gold nanoparticles into HEp-2 cells after, a) 2 h, b) 4 h, c) 12 h, d) 24 h incubations.

3.2.3. Atomic Force Microscopy Study

Our AFM data also corresponded with other microscopy studies discussed above. After 1 h incubation, 3, 10 and 26 nm gold nanoparticles entered to nucleus, whereas 50nm particles highly accumulated around nucleus. Our three microscopy studies showed that although 50 nm particles are taken intracellularly by receptor-mediated endocytosis pathway (5), these particles have been aborted by nuclear pore complexes, after 1 h incubation.

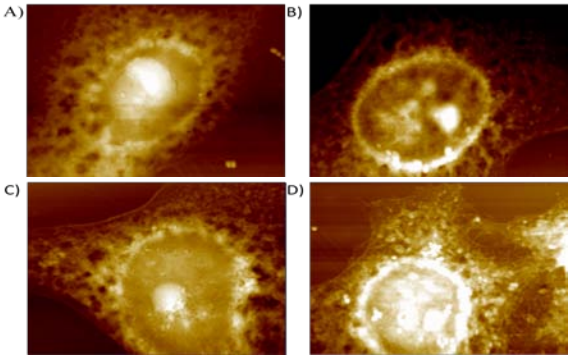

Figure 6. AFM imaging of A) 3 nm, B) 10 nm, C) 26 nm, and D) 50 nm GNPs into HEp-2 cells after 1 h incubation.

The following time interval studies showed that all size of GNPs localized into the nucleus. These results indicated that after 2 h, and following incubation times, GNPs get located into cell nucleus without any conjugation.

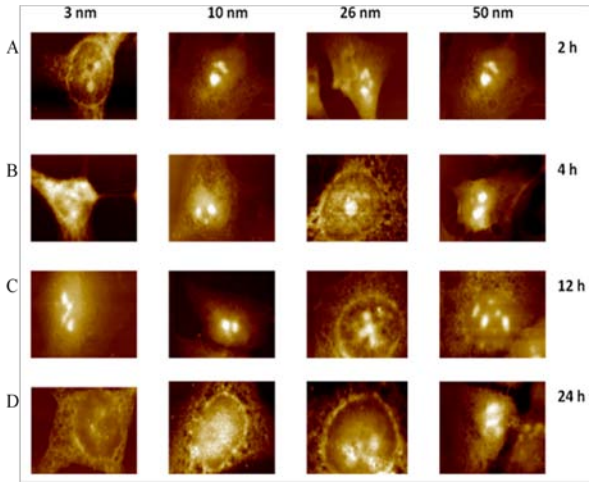

Figure 7. AFM imaging of 3, 10, 26, and 50 nm gold nanoparticles into HEp-2 cells after, a) 2 h, b) 4 h, c) 12 h, d) 24 h incubations. We also observed GNPs into nucleus at later time intervals.

In this study, we investigated the scientific understanding of intracellular uptake of GNPs *in vitro*. We started our study with measuring the toxicity level of GNPs using MTS assay. Our MTS showed that smallest size GNPs, 3 nm in diameter, exhibited highest toxicity level, thereby suggesting that our results corresponds with the most of previous studies (6). In these studies, it has been reported that small particles are more suitable to interact with DNA than larger particles, which causes distruption in the cells.

We continued our study with different microscopic techniques, including SEM, AFM and confocal microscopy. Our data showed that the most of GNPs have entered cell nucleus at different rates depending on the particle sizes. SEM and AFM results showed that after 1 h incubation, 3, 10 and 26 nm GNPs entered into the nucleus, whereas 50 nm particles highly accumulated around nucleus. These results corresponded with confocal microscope images, which indicated that single GNPs emitted blue color, whereas their aggregation sides leads to emission in red color range. Our data confirmed that small nanoparticles emit energy with small wavelength (λ), whereas particles with larger surfaces release energy of larger wavelength. As GNPs enter cells, they accumulated in nucleus, resulting in the emission of energy with larger wavelength. Thus, we observed aggregated particles with red color fluorescence. As consequence, we suggest that that 3 nm GNPs with sphere shape may be appropriate candidate in the field of gene and drug delivery applications, whereas larger particles are more suitable for anti-viral applications, specifically, for the inhibition of DNA viruses even without any conjugation.

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