Influence of Cell Spreading Area on Uptake of Gold Nanoparticles

Adriana Mulero
Chemical Engineering, University of Puerto Rico-Mayaguez

NNIN iREU Site: National Institute for Materials Science (NIMS), Tsukuba, Ibaraki, Japan
NNIN iREU Principal Investigator: Dr. Guoping Chen, Tissue Regeneration Materials Unit, National Institute for Material Science
NNIN iREU Mentor: Xinlong Wang, Tissue Regeneration Materials Unit, National Institute for Material Science
Contact: adriana.mulero1@upr.edu, guoping.chen@nims.go.jp, wang.xinlong@nims.go.jp

Abstract:

Nanoparticles (NPs) have been proved to be a promising material for drug delivery, cancer therapy, and bioimaging. However, there is growing concern about their cytotoxicity, which may cause an inverse effect on human health. In order to develop safer and efficient NPs, people need to fully understand how cells react with the NPs. Previous studies investigated the influence of NPs properties on cellular uptake. However, little attention was paid to understand the influence of cell membrane tension on the uptake. Micropatterns were used to control the membrane's tension on single cell scale; the greater the spreading area, the greater the tension on the cell membrane [1]. Thus in this study, a serious of microdots with various diameters (20, 40, 60, and 80 μm) were developed using photolithography techniques. Gold nanoparticles (AuNPs) with sizes of 50 nm were synthesized following the Turkevich method and coated with FITC labeled PEG. Mesenchymal stem cells (MSCs) were cultured on the micropatterns and treated with FITC-PEG-AuNP to investigate the influence of cell spreading area on uptake of NPs. The results indicate that PEG-coated AuNP are non-toxic to the MSCs. Patterned cells with different spreading areas and cellular tension can uptake the FITC-PEG AuNPs.

Introduction:

Nanoparticles (NPs) have been found to be a promising way for visualizing specific cell types, delivering drugs directly into the cell, and cancer therapies. For all these applications to be successful, it is important to study the NPs and cell interaction, including cellular uptake and cellular toxicity. Endocytosis is the mechanism that cells use to bring the nutrients and NPs into the cell. The cellular membrane bends forming a vesicle, providing them transportation into the cell. Previous studies [1] have analyzed NPs properties and their effects on the uptake. However, little attention was paid to understand how the cellular membrane properties influence the cellular uptake. These include the cellular membrane tension affecting the membrane's capacity to bend to form the vesicles.

Micropatterns have been proved to efficiently control the cellular membrane tension on a single cell scale; the greater the spreading area, the greater the tension on the membrane [2]. In this project, we evaluated how the cellular membrane tension, of mesenchymal stem cells (MSCs), controlled by micropatterns of different size dots affects the uptake of gold nanoparticles (AuNP).

Experimental Procedure:

Micropatterning Process. By using photolithography techniques, the micropatterns were developed on a transparent polystyrene square surface (6.25 cm^2) cut from a tissue culture dish, which supported cell adhesion. Poly(vinyl alcohol) (0.3 mg/mL) was used as the photoreactive solution. The design consisted of dots with diameters of 20, 40, 60, and 80 μm. The micropatterned surfaces were characterized using atomic force microscopy.

AuNP Synthesis. Following the Turkevich method, we synthesized the 50 nm sphere AuNP [3] using 0.01% chloauric acid ($HAuCl_4$) and 1% tri-sodium citrate solution as the reduction solution. The synthesis was kept at constant stirring and a temperature of 110°C. It was later ultrasoniced to disperse the molecules before the surface modification. The mPEG-SH and the FITC-PEG-SH were added to AuNP solution at a 3:2 ratio. The mix was stirred overnight to obtain the FITC-PEG modified AuNP.

Cell Experiments. The cell experiments were divided into two groups: flat and patterned surface. For the flat surface, we seeded the cells on a 96-well-plate with a cell density of 1,000 cells/well. For the patterned surface, the micropatterns were sterilized with ethanol and water, then placed one square per well on a six-well-plate; cell density 5,000 cells/well. After the cells attached, the previous medium was aspirated and the growth medium containing the AuNPs was added to the cells. They were treated with 0.1, 0.5, 1 and 3 nMFITC-PEG AuNP concentrations. The cells were then tested for cell viability with WST-1, and cellular uptake studied with fluorescence microscope.

Results and Conclusions:

Micropatterns were characterized to measure the diameter and depth of each pattern. Patterns were proved to go according to the design, with a depth around 60 nm (Figure 1). Nanoparticles were characterized with scanning and transmission electron microscope to analyze their shape and size distribution (Figure 2). Size was proved to be spherical, and from the increase in size, we said that FITC-PEG layer was successfully added. Cell viability test (WST-1), was performed 6, 12, and 24 hours post-seeding. The WST-1 results showed no statistical difference between the NPs treated group and the control group (Figure 3). For the NPs uptake (Figure 4), cells were examined under the fluorescence microscope to view the NPs fluorescence due to the FITC modification.

On the bright field, the NPs were observed with a red color, pointed with arrows. Whenever the NPs were aggregated, the green fluorescence intensity increases. The nucleus, stained blue, showed there was one cell per dot. There is green fluorescence inside the contour of the cell every size dot, suggesting there are NPs inside the cells.

We concluded the following. First, the FITC-PEG AuNP was non-toxic to MSCs, and they presented a promising way to visualize cellular uptake. Second, the patterned cells with different spreading areas, and cellular tension could uptake FITC-PEG AuNPs. Future work includes quantifying the fluorescence intensity to obtain quantitative NPs uptake results and visualizing the NPs distribution inside cells using laser confocal microscope.

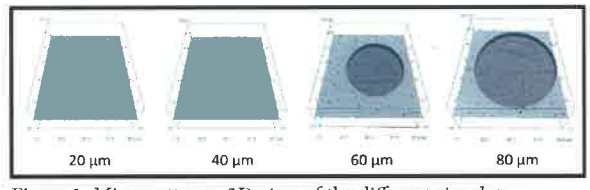

Figure 1: Micropatterns, 3D view of the different size dots.

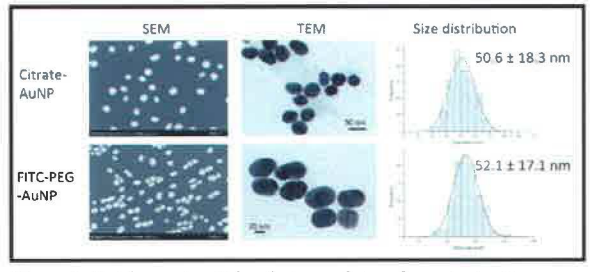

Figure 2: Gold nanoparticles shape and size characterization.

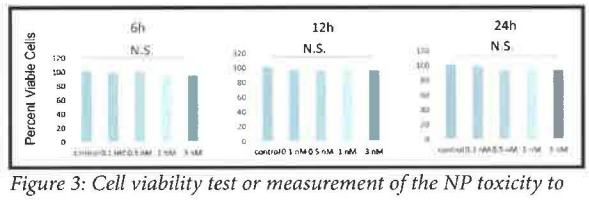

Figure 3: Cell viability test or measurement of the NP toxicity to cells.

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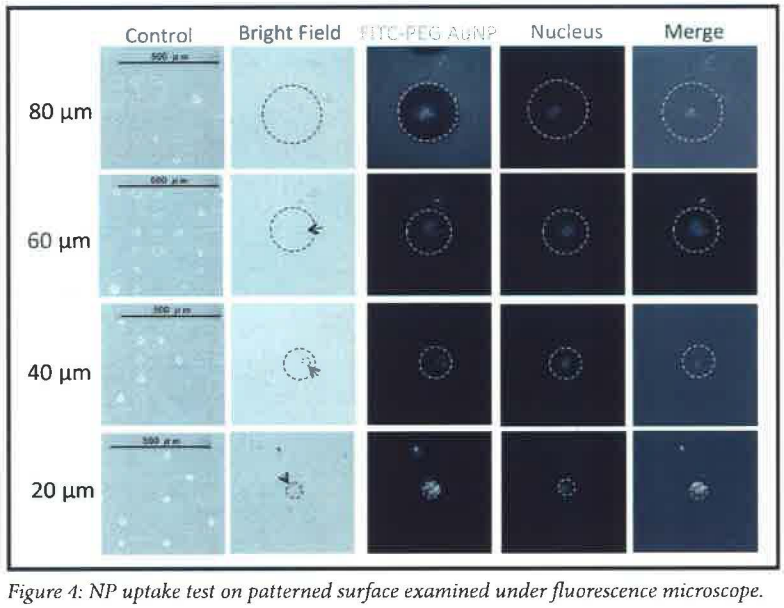

Figure 4: NP uptake test on patterned surface examined under fluorescence microscope.

References:

[1] ACS Nano, 7, 1961, 2013. J. Colloid Interface Sci., 438, 68, 2015. Biomaterials, 54, 226, 2015.

[2] Biophysical Journal, 94, 4984, 2008.

[3] Environmental Toxicology and Chemistry, 33, 481, 2014.