The Effects of Chemical and Biological Gold Nanoparticles on Human Dermal Fibroblasts

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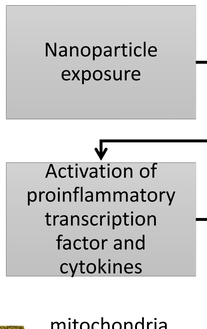
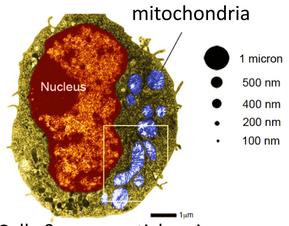
OFFICE OF UNDERGRADUATE RESEARCH AND INQUIRY

Introduction

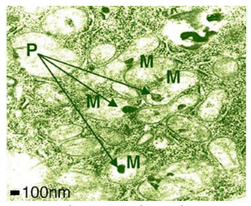
- Nanoparticles (NPs) are derived from bulk chemicals via chemical reduction, which allows them to have electromagnetic, optical, antimicrobial, and stable components[1]
- Chemical reduction methods raise concern for cytotoxicity
- NPs have potential to be chemically synthesized or biologically synthesized, where each may have different cytotoxic effects based on size, crystalline structure, and shape
- Biologically synthesized NPs are environmentally friendly, cost effective, and do not require a toxic reducing agent[2,3]
- Gold NPs are used in skincare and cosmetic products, so this study analyzed the cytotoxic effect on human dermal fibroblasts

Why study cytotoxicity?





Cells & nanoparticles size.



NPs inside mitochondria

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Our Approach

Synthesis and characterization of biological gold nanoparticles (AuNPs)

Reducing agent: *Camellia sinensi* (black tea) extract
Reductant: 0.5 mmol or 1 mmol Gold(III) chloride trihydrate ($HAuCl_4 \bullet 3H_2O$)

Nanoparticle characterization:
-Transmission electron microscopy
-Zeta potential Analysis
-Dynamic Light Scattering

Toxicity of citrate capped AuNPs and black tea extract synthesized biological AuNPs (18-20 nm)

Cell line: human primary dermal fibroblasts (HDF)
Temporal studies: 24, 48, and 72 hour analysis
AuNP concentrations: 0, 5, 10, 25, 50, and 100 µg/mL AuNPs

Cytotoxicity analysis:
-MTT and LDH assay
-Flow cytometry analysis
-Immunofluorescence staining with DAPI and Phalloidin

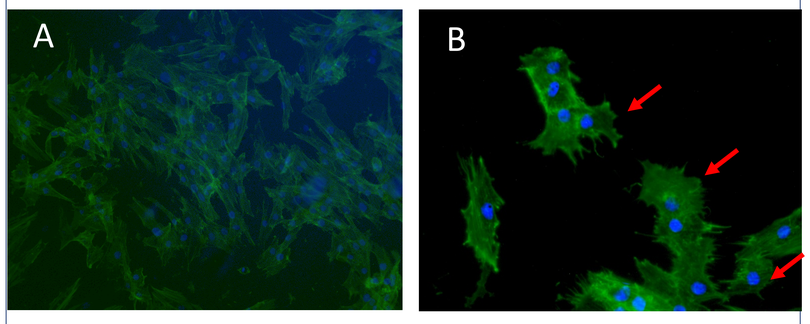

Figure 1. Immunofluorescence microscopy images using DAPI and phalloidin staining on human dermal fibroblast cells in different concentrations of AuNPs A) Control, no nanoparticle solution. B) 50 µg/ml AuNP (Bio). Phalloidin (F-actin) was disrupted in a few cells (red arrow) at 50 µg/ml AuNP.

Results

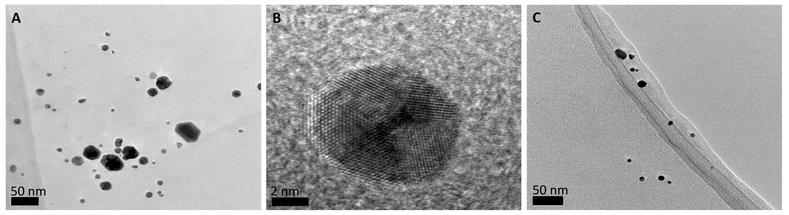

Figure 2. TEM images of AuNPs using black tea extract. A)AuNPs synthesized with 1 mmol $HAuCl_4$. B) HR-TEM of AuNPs using 0.5 mmol $HAuCl_4$ showed a d-spacing of 0.235 nm, suggesting (111) plane of crystalline AuNPs. C) AuNPs synthesized using 0.5 mmol $HAuCl_4$.

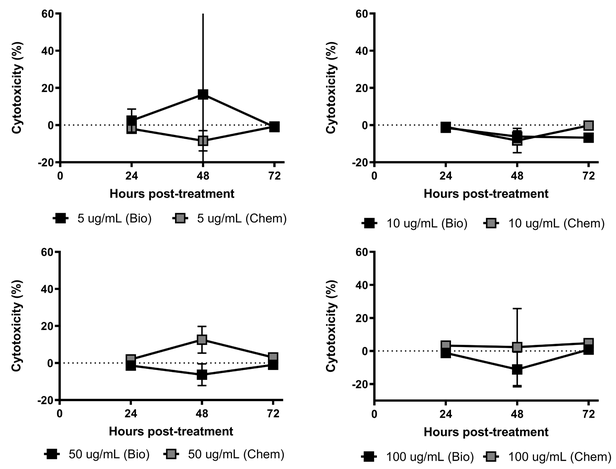

Figure 3. LDH Assay percent cytotoxicity results with 5, 10, 50, and 100 µg/mL of biological and chemical AuNPs at 24, 48, and 72 hours post AuNP treatment.

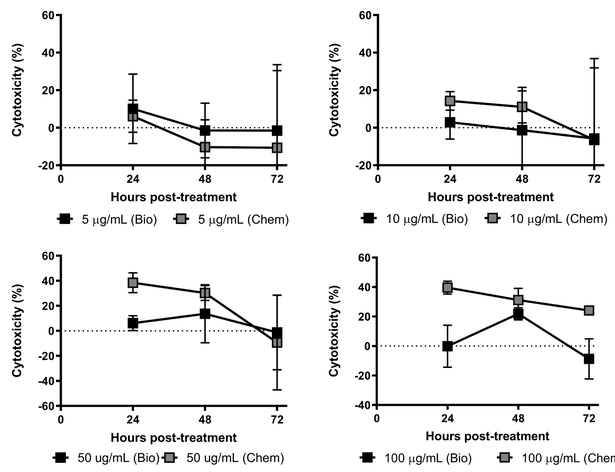

Figure 4. MTT Assay percent cytotoxicity results with 5, 10, 50, and 100 µg/mL of biological and chemical AuNPs at 24, 48, and 72 hours post AuNP treatment.

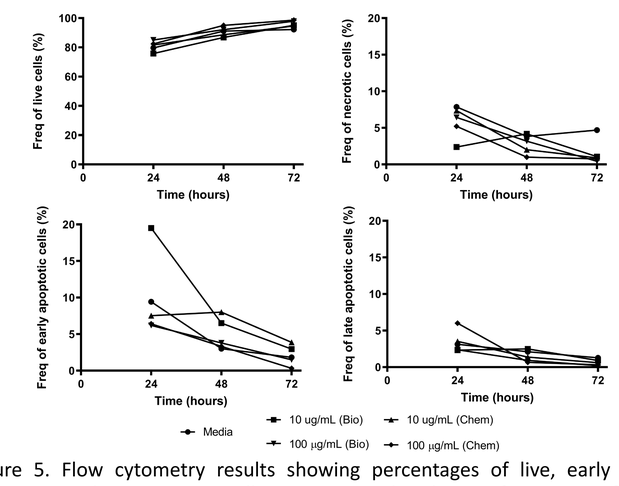

Figure 5. Flow cytometry results showing percentages of live, early apoptotic, apoptotic, and necrotic HDF cells at 24, 48, and 72 hours of exposure of 10 or 100 µg/mL biological and chemical AuNPs.

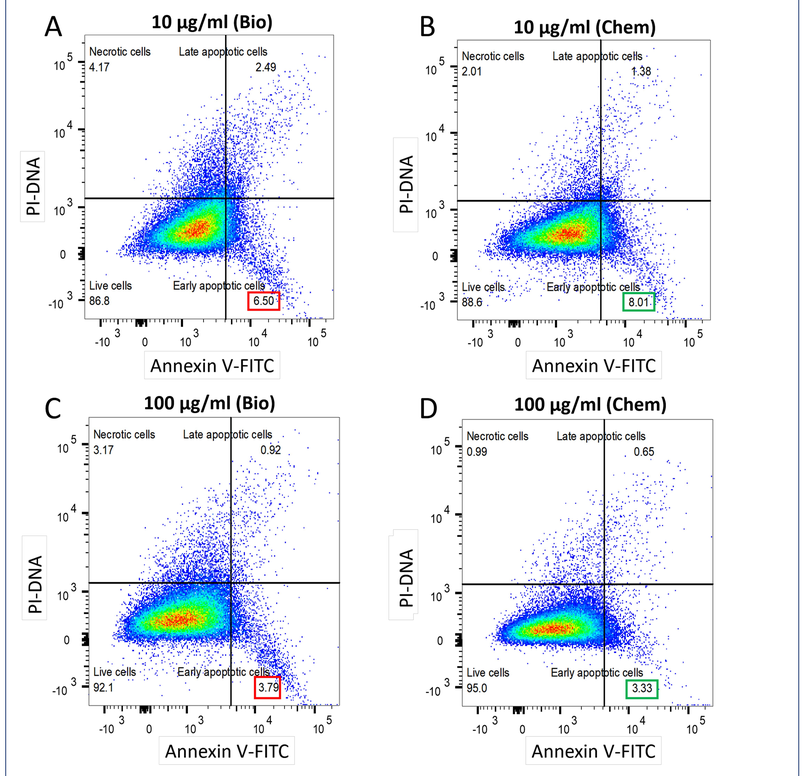

Figure 6. Flow cytometry results of 48 hour exposure of HDF cells to biological and chemical AuNPs. A) 10 µg/mL biological AuNP B) 10 µg/mL chemical AuNP C) 100 µg/mL biological AuNP D) 100 µg/mL chemical AuNP. At lower concentrations (10 µg/ml), a higher population of early apoptotic cells were seen compared to higher concentrations (100 µg/ml) of AuNPs.

Discussion

- 0.5 mM $HAuCl_4 \bullet 3H_2O$ biological AuNP solution produced optimal NPs of a narrow range particle size distribution (5-18 nanometers in size).
- HR-TEM showed the presence of crystalline gold nanoparticles.
- Zeta potential analysis showed the a broader distribution of charges on the surface of chemical AuNPs than biological AuNPs.
- F-actin disruption was seen at higher doses (50 µg/ml) of AuNP exposure.
- At higher dose (100 µg/ml), cells may recover from early and late apoptotic stages leading to a higher population of live cells.
- LDH and MTT assay showed slight decrease in cell viability/toxicity over a period of 72 hrs.
- Overall, there was no significant difference in cytotoxicity for both biological and chemical AuNPs.
- Flow cytometry analysis by FlowJo showed an increase in the number of live cells after 72 hours of exposure to both chemical and biological AuNPs at 100 µg/ml.

References

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Acknowledgements

We would like to thank University of Tampa Biology Department and the University of South Florida Morsani College of Medicine for allowing us to use their facilities, University of Tampa's Office of Undergraduate Research and Inquiry for the grant to conduct this study, Pavan Rajanahalli for cell culture training, Sai Lata De for flow cytometry, and Yusuf Emirov for TEM analysis.