Introduction of Gold Ion Solution into Living Cells: Uptake Mechanism and Applications

P. Thompson, S. Fujii, N. Smith, K. Fujita, S. Kawata

Laboratory for Scientific Instrumentation and Engineering, Osaka University, Suita, Japan

A better understanding of the relationship between living cells and nanotechnology is essential for successful clinical applications. By monitoring the response of a cell colony after the introduction and uptake of a gold ion solution, it may be possible to determine and predict the ideal conditions for a wide array of techniques, ranging from an improvement in imaging systems such as Raman spectroscopy to potent cancer treatment therapies. In this series of experiments, living HeLa cells in Petri dishes were cultured in Dulbecco/Vogt Modified Eagle's Minimum Essential Medium (DMEM) until covering 30-40% of the dish. At this point, the medium was changed and gold solution was added. In each test, one of three different media (each modifications of a basic saline solution) and one of three different concentrations of gold (III) chloride were combined and introduced to the cells. In order to determine the most effective combination for gold formation inside the cell, dishes were monitored for 96 hours. Every 24 hours, cells were tested for viability (using fluorescence microscopy) and gold formation within the cell (using dark field microscopy). Preliminary results suggest that successful cellular uptake of gold ion solution is not dependent upon concentration or media, and that cells internalize the solution within 24 hours. Additionally, higher concentrations of gold ions in solution do not seem to cause any notable changes in cell survival over a 96 hour period when compared to a control set without gold ions. However, when a lower concentration of gold ions is used, cell survival is adversely affected.

Introduction of Gold Ion Solution into Living Cells: Uptake Mechanism and Applications

Paul Thompson (paul.thompson@rice.edu), Shintaro Fujii, Nicholas Smith, Katsumasa Fujita, Satoshi Kawata

Laboratory for Scientific Instrumentation and Engineering, Kawata Laboratory, Osaka University, Suita Campus

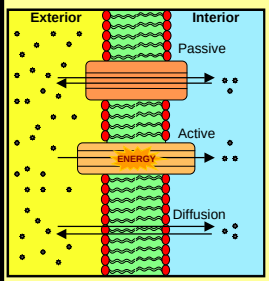
Introduction

•A better understanding of the relationship between living cells and nanotechnology is essential for successful clinical applications.

•By closely monitoring the response of a cell colony after the introduction and uptake of a gold ion solution, it may be possible to determine and predict the ideal conditions for a wide array of scientific techniques.

- •Improvement in assorted imaging systems such as Raman spectroscopy
- •Potent cancer treatment therapies.
- •Advanced exploration of cellular biology.

Fundamentals and Theory

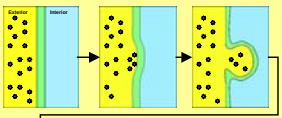


Membrane Transport

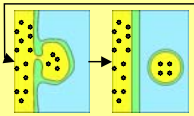
•The cell membrane controls the flow of nutrients in and out of the cell by using a number of different transportation pathways.

- •Passive transport mediated by membrane proteins
- •Active transport (requires energy)
- •Diffusion across selectively permeable membrane

•Endocytosis involves internalizing portions of the cell membrane, drawing external particles inside



•Gold ion solution uptake may occur through a combination of these pathways


Endocytosis

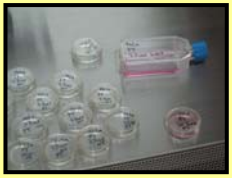
Questions

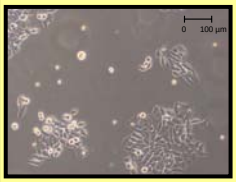
•Can living cells survive in gold ion solution, and, if so, what is the best combination of gold ion concentration and cellular medium for survival?

•Can gold ions be reduced inside the cell and form gold nanoparticles?

Methods

•HeLa (human cervical cancer) cells were cultured in glass-bottom dishes using Dulbecco/Vogt Modified Eagle's Minimum Essential Medium (DMEM) for cellular nutrition, then incubated at 37 degrees Celsius and 5 percent CO_2 for approximately 48 hours.


Colony of HeLa cells and dishes



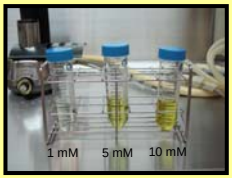
HeLa cells at approximately 30 percent confluency

•When dishes were 30-40 percent confluent (covered with cells), DMEM was exchanged with experimental media and gold (III) chloride ($HAuCl_4$) was added.

•Media was either Phosphate Buffered Saline (PBS) with glucose, PBS without glucose, or DMEM without pH indicator (colorless).

•Gold (III) chloride solutions were in concentrations of 1 mM, 5 mM, or 10 mM.

•Control dishes without any gold (III) chloride were also prepared, to evaluate the effect of gold on cell viability.



Three different concentrations of gold (III) chloride solutions


Fluorescence Microscope

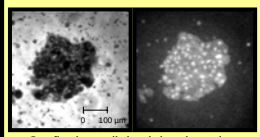
•After incubation for approximately 48 hours, dishes were monitored for 96 hours.

•Every 24 hours, cells were checked for viability (using propidium iodide (PI) indicator and fluorescence microscopy).

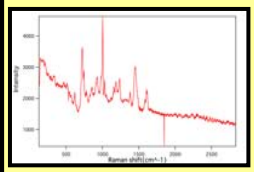
•In addition, time lapse photography was used in conjunction with dark field microscopy for periods of two or three hours to see whether gold uptake was visible over an extended period of time.

•Finally, after 48 hours of incubation with gold ion solution, Raman spectroscopy was used with the most successful results from above to confirm the formation of gold within the cells.

Results



Confirming cell death by observing fluorescence during PI test


Raman spectra obtained from 1 mM $HAuCl_4$ in PBS without glucose

•In cases involving PBS with and without glucose, cells incubated with $HAuCl_4$ survived longer (approx. 24 hours) than controls.

•Although time lapse photography could not confirm the movement of $HAuCl_4$ into the cells, preliminary Raman spectra results suggest that gold ion solution was internalized by the cells incubated with 1 mM $HAuCl_4$ in PBS without glucose.

Further Research and Applications

•The Laboratory for Scientific Instrumentation and Engineering plans to continue research on the effect that gold ion solution has on cellular viability.

- • An expanded version of the trial will be conducted to facilitate easier and more accurate comparisons between experimental sets and controls.
- •In addition, a wider variety of cell media and $HAuCl_4$ concentrations will be used to glean more information about the mechanisms behind experimental results.
- •Transmission electron microscopy will be used to obtain additional images of gold particles within cells.

•Research may eventually result in an effective form of intracellular targeted photodynamic therapy.

Acknowledgements

•The NanoJapan Program is generously funded by the National Science Foundation. Many thanks to LaSIE at Osaka University's Suita Campus for allowing me to conduct research with their group and Rice University for giving me this opportunity.

References

1. Anshup, J.S.V., Subramaniam, C., Rajeev Kumar, R., Priya, S., Santhosh Kumar, T.R., Omkumar, R.V., John, A. and Pradeep, T. (2005). Growth of Gold Nanoparticles in Human Cells. *Langmuir* 21, 11562-11567.
2. Glomm, W.R. (2005). Functionalized Gold Nanoparticles for Applications in Bionanotechnology. *Journal of Dispersion Science and Technology* 26, 389-414.
3. Shamsaie, A., Jonczyk, M., Sturgis, J., Paul Robinson, J., and Irudayaraj, J. (2007). Intracellularly grown gold nanoparticles as potential surface-enhanced Raman scattering probes. *Journal of Biomedical Optics* 12 (2), 020502-1-020502-3.