

ICP - Mass Spectrometry

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Quantification of Gold Nanoparticle Uptake into Cancer Cells using Single Cell ICP-MS

common cause of death in the US.¹ Current treatments for various cancers include surgery, radiation, immunotherapy, and chemotherapy. Although these conventional therapies may improve patients' overall survival and quality of life, they also have several limitations. For example, in conventional cancer chemotherapy, small-molecule-based cancer therapeutics distribute non-specifically throughout the entire human body. The consequence is that these drugs do not only kill cancer cells but also destroy healthy cells in the body causing severe side effects for cancer patients,² leading to a need for new therapies that can target diseased cells.

Nanoparticles (NPs) have been of significant interest over the last two decades as they offer attractive benefits for drug delivery to overcome limitations in conventional chemotherapy.³ Nanoparticles can be engineered to carry both drugs and imaging probes to simultaneously detect and treat cancer. They may also be designed to specifically target diseased tissues and cells in the body. A number of nanoparticle-based cancer therapeutics have been approved for clinical use and/or are currently under development.^{4,5} Advantages that rationally engineered nanoparticles may offer over conventional small-molecule drugs include: (i) prolonged circulation time in the body; (ii) reduction of nonspecific cellular uptake and undesirable off-target and side effects; and (iii) improvement in cellular interactions through specific cancer cell targeting moieties.

Introduction

Cancer is a disease that is characterized by the uncontrolled growth and spread of abnormal cells. According to the American Cancer Society, cancer is the second most

The therapeutic effect of cancer treatment is related to the amount of drug that interacts with each individual cancer cell. Traditional drug research techniques for measuring drug exposure, such as conventional inductively coupled plasma mass spectrometry (ICP-MS), have been limited to cell ensemble measurements. However, these measurements require homogenization of a given cell population for quantitative analysis. All cells within this population are assumed to be similar and, therefore, assumed to interact with the same amount of drug. In contrast, studies demonstrate that cell populations are heterogeneous, and differences exist even for cells from the same cell population and cell line.⁶ For example, gene expression measurements based on homogenized cell populations may be misleading as they only provide averaged results and do not account for the small, but critical, changes occurring in individual cells. Individual cells can differ significantly in size, protein levels, and expressed ribonucleic acid (RNA) transcripts. These variations are key aspects when answering previously unsolvable questions in cancer research, stem cell biology, immunology, developmental biology, and neurology.

To overcome these limitations that arise from batch analysis approaches, Single Cell (SC) ICP-MS has been developed. This technique allows the rapid and robust analysis of a large number of individual cells rather than a cell population as a whole or only a few cells.⁷ Studies have demonstrated both the ability to measure metallic components within cells and quantify the cellular uptake rate of metals, metal-doped drugs, and nanoparticles using SC-ICP-MS.⁸⁻¹¹

In this work, we demonstrate the use of PerkinElmer's NexION[®] 2000 Single Cell ICP-MS solution to quantify the uptake of gold nanoparticles into cancer cells on an individual cell basis. This technique is able to precisely quantify the number of cells containing gold nanoparticles (AuNPs), as well as the number of AuNPs in each individual cancer cell analyzed, giving a distribution of uptake in the cell population.

Experimental

Samples and Sample Preparation

Citrate-stabilized 50 nm AuNPs were prepared using a seed-mediated synthesis strategy as reported by Perrault and Chan.¹² To increase colloidal stability in tissue culture media, AuNPs were PEGylated at room temperature with 5 kDa methoxy-terminated thiol poly(ethylene glycol) (mPEG_{5kDA}-SH, Laysan Bio, USA) at a ratio of 40,000 mPEG-SH molecules per AuNP in deionized (DI) water. Dynamic light scattering (DLS) experiments of AuNPs were conducted using a Malvern ZetaSizer Nano ZS, and a Hitachi H-7600 transmission electron microscope (TEM) equipped with a Kodak 2Kx2K digital camera which was used to acquire electron micrographs of AuNPs.

Human T24 urinary bladder cancer cells were purchased from ATCC (www.atcc.org). Cells were grown as monolayers on tissue-culture-treated petri dishes with McCoy's 5A media (supplemented with 10% FBS). For the AuNPs uptake experiment, T24 cancer cells were seeded on

tissue-culture-treated 6-well plates at a density of one million cells per well and incubated at 37 °C (5% CO₂) for 24 h to ensure adhesion onto the tissue culture plate surface. The cells were then exposed to PEGylated 50 nm AuNPs (gold core diameter) at a concentration of 0.4 nM of AuNPs in McCoy's 5A media (supplemented with 10% FBS) for 4 h. Next, the cells were washed with 1xPBS five times, enzymatically detached from the tissue culture plate surface, and fixed with 4% paraformaldehyde in 1xPBS to form a single cell suspension with a final concentration of 100,000 cells/mL.

Instrumentation

All analyses were done on a NexION 2000 ICP-MS running the Single Cell Application Module in Syngistix software using the conditions in Table 1. The components used for SC-ICP-MS analysis are shown in Figure 1 and include the NexION 2000 Asperon™ spray chamber as well as the specialized Single Cell Micro DX Autosampler. The Asperon spray chamber is designed to maximize cell transport to the NexION, while the Single Cell Micro DX Autosampler agitates the cellular suspensions prior to analysis to ensure that the cells have not settled out of solution.

Table 1. NexION 2000 ICP-MS Instrumental Conditions.

Parameter/Component	Value
Nebulizer	MEINHARD [®] High Efficiency Nebulizer (HEN)
Spray Chamber	Asperon
Make up Gas (L/min)	0.7
Neb Gas (L/min)	0.54
Plasma Power (W)	1600
Sample Flow Rate (μL/min)	10
Autosampler	Single Cell Micro DX
Agitation	Single Cell Micro DX Autosampler
Sample Loop (μL)	50



Figure 1. Hardware components for Single Cell ICP-MS: NexION 2000 ICP-MS (A), Asperon spray chamber (B), Single Cell Micro DX Autosampler (C).

Results and Discussion

Nanoparticle Characterization

Colloidal dispersions of citrate-stabilized AuNPs were prepared according to a reported protocol.¹² A transmission electron micrograph of these AuNPs (Figure 2) shows that they were monodisperse with an average diameter of 50 nm and spherical shape. The diameter of these nanoparticles was further confirmed by Single Particle ICP-MS (SP-ICP-MS) also using the NexION 2000. The results of this analysis are shown in Figure 3.

Citrate-stabilized AuNPs were surface modified with mPEG_{5kDA}-SH to improve colloidal stability in tissue culture media and used for nanoparticle-cell uptake experiments. Dynamic light scattering (DLS) experiments of PEGylated AuNPs revealed a hydrodynamic diameter of 79.9 ± 2.3 nm with a polydispersity index (PDI) of 0.056 after surface modification (Figure 4). Whereas the TEM and SP-ICP-MS results are comparable, due to the fact they both measure the AuNP metallic core diameter, the DLS results show larger sizes. The DLS results are based on the Brownian motion of the AuNPs in dispersion and thus include the length of the polymer ligands surrounding the AuNPs. This means that the DLS results indicate larger NP sizes that take into account the hydrodynamic diameter. The zeta potential of these PEGylated AuNPs in DI water is close to neutral (data not shown).

Nanoparticle characterization experiments confirmed that AuNPs were monodisperse, colloidal stable, and of high quality, which are prerequisites for downstream quantification of AuNP uptake into cancer cells using SC-ICP-MS.

Uptake into T24 Cancer Cells

Human T24 urinary bladder cancer cells were used as model cancer cells to quantify the cellular nanoparticle uptake upon exposure to PEGylated AuNPs. T24 cancer cells were incubated with 0.4 nM of PEGylated AuNPs for 4 h. After extensive washing with 1xPBS to remove excess AuNPs, T24 cancer cells were fixed with 4% paraformaldehyde to form a single cell suspension. The number of AuNPs per cell was quantified, and results from SC-ICP-MS analysis revealed the distribution of AuNPs per cell ranging from one to five AuNPs/cell (Figure 5).

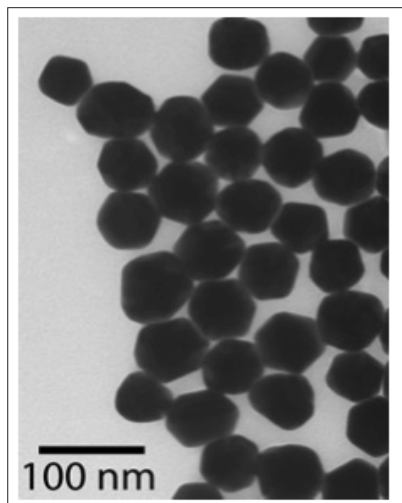


Figure 2. Transmission electron micrograph of 50-nm AuNPs. The image shows that AuNPs exhibit a spherical shape with an average diameter of approximately 50 nm.

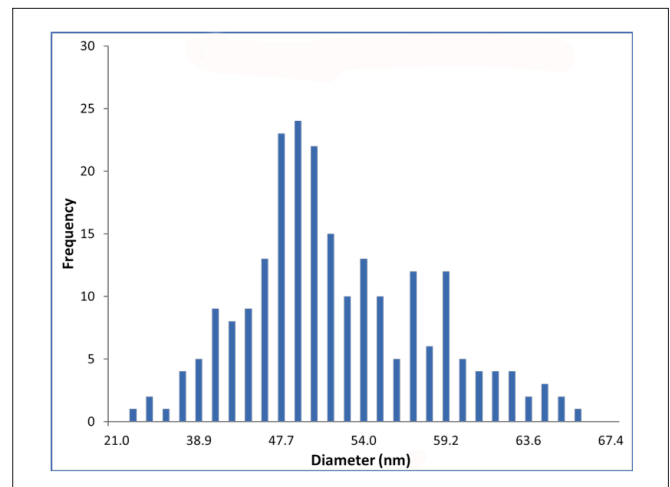


Figure 3. Gold nanoparticle size distribution analysis based on Single Particle ICP-MS (SP-ICP-MS).

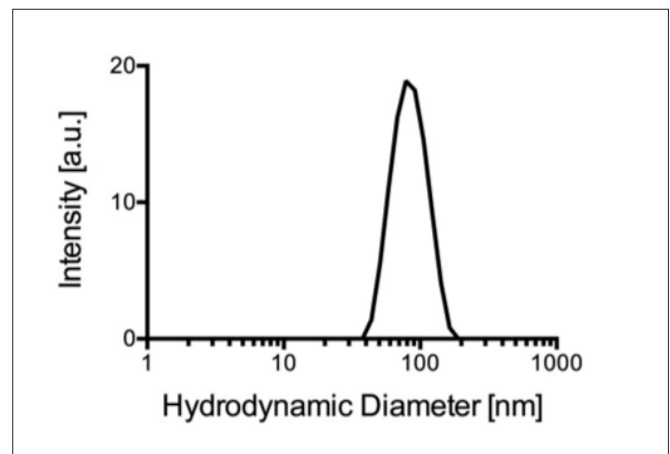


Figure 4. Dynamic light scattering (DLS) characterization of PEGylated AuNPs in DI water. The hydrodynamic diameter of PEGylated AuNPs is 79.9 ± 2.3 nm (PDI 0.056).

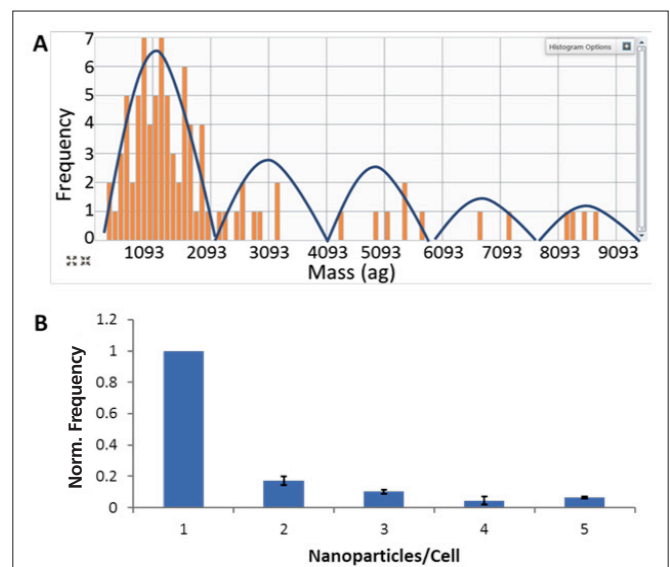


Figure 5. SC-ICP-MS analysis of human bladder cancer cells (T24) incubated with PEGylated 50-nm (gold core size) AuNPs for 4 h, using a PerkinElmer NexION system. (A) Histogram of gold mass distribution for individual T24 cells – blue curves highlight populations of cells containing one or more AuNPs per cell. (B) Normalized frequency distribution histogram of cells containing one or more AuNPs (norm. to number of cells containing one AuNP; $n=3$) – bars indicate average values with standard deviations.

SC-ICP-MS analysis allows the quantification of the number of AuNPs taken up by cancer cells on an individual cell basis. This type of data is unique and cannot be obtained from conventional ensemble-based ICP-MS approaches. Our results indicate that uptake of AuNPs into cells is not homogeneous in terms of number of nanoparticles per cell as assumed when using conventional digestion methods. The preparation of a homogeneous cell suspension and subsequent quantification of gold content using a conventional ICP-MS approach suggests that each cancer cell contains on average 1.5 ± 0.1 AuNPs per cell. However, this result does not provide information on how individual cells interact with AuNPs.

Based on the data shown in Figure 5, a logical next step is to investigate the reasons and potential biological mechanisms for the observed nanoparticle cell distributions using SC-ICP-MS. In other words, why do certain cells uptake more AuNPs than others? This is important as AuNPs are promising nanomaterials with increasing use for diagnostic and therapeutic applications in cancer management.^{4,13} Quantitative information about how AuNPs interact with individual cancer cells has been limited in the literature due to a lack of available analytical tools. SC-ICP-MS (a technology that allows introduction of intact individual cells into a highly sensitive ICP-MS) provides access to this important information by quantifying intracellular elemental concentrations on a single cell basis – one intact cell at a time.

Conclusion

In this work, we quantified AuNP uptake into cancer cells on an individual cell level using Single Cell ICP-MS. Our results showed that the distribution of AuNPs per cell is heterogeneous and that certain cells within a given cell population uptake significantly more AuNPs than others. SC-ICP-MS is a powerful analytical tool that allows quantification of elemental concentrations and nanoparticle distributions on an individual cell level. This technique has the potential to provide new insights in the differences of nanoparticle-cell interactions on a single cell basis.

References

1. <https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2018/cancer-facts-and-figures-2018.pdf>
2. Love, R.R., Leventhal, H., Easterling, D.V., and Nerenz, D.R. (1989). "Side effects and emotional distress during cancer chemotherapy." *Cancer*, 63(3), 604-612.
3. Peer, D., Karp, J.M., Hong, S., Farokhzad, O.C., Margalit, R., Langer, R. "Nanocarriers as an emerging platform for cancer therapy." *Nature Nanotechnology* 2, no. 12 (2007): 751.
4. Shi, J., Kantoff, P.W., Wooster, R., and Farokhzad, O.C. (2017). "Cancer nanomedicine: progress, challenges and opportunities", *Nature Reviews Cancer*, 17(1), 20.
5. Wilhelm, Stefan, Tavares, A.J, Chan, W. "Reply to "Evaluation of nanomedicines: stick to the basics". " *Nature Reviews Materials* 1, no. 10 (2016): 16074.
6. Magee, J.A., Piskounova, E., Morrison S.J. "Cancer stem cells: impact, heterogeneity, and uncertainty." *Cancer Cell* 21, no. 3 (2012): 283-296.
7. "Single Cell ICP-MS Analysis: Quantification of Metal Content at the Cellular Level", PerkinElmer, 2017.
8. "Monitoring the Uptake of Nanoparticles and Ionic/ Dissolved Gold by Fresh Water Algae using Single Cell ICP-MS", PerkinElmer, 2017.
9. "New Research Evaluating Cisplatin Uptake in Ovarian Cancer Cells by Single Cell ICP-MS", PerkinElmer, 2017.
10. "Iron Content Measurement in Individual Bacterial Cells Using SC-ICP-MS", PerkinElmer, 2018.
11. Merrifield, R., Amable, L., Stephan, C. "Single Cell ICP-MS Analysis: Quantifying the Metal Concentration of Unicellular Organisms at the Cellular Level", *Spectroscopy*, 33 (6), 33-39.
12. Perrault, S.D., and Chan, W.C. (2009). "Synthesis and surface modification of highly monodispersed, spherical gold nanoparticles of 50–200 nm", *Journal of the American Chemical Society*, 131(47), 17042-17043.
13. Wilhelm, S., Tavares, A.J., Dai, Q., Ohta, S., Audet, J., Dvorak, H.F., and Chan, W.C. (2016). "Analysis of nanoparticle delivery to tumours", *Nature Reviews Materials*, 1(5), 16014.