Cell Mass Spectrometry

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Quantitative Measurement of Nano-/Microparticle Endocytosis by Cell Mass Spectrometry**

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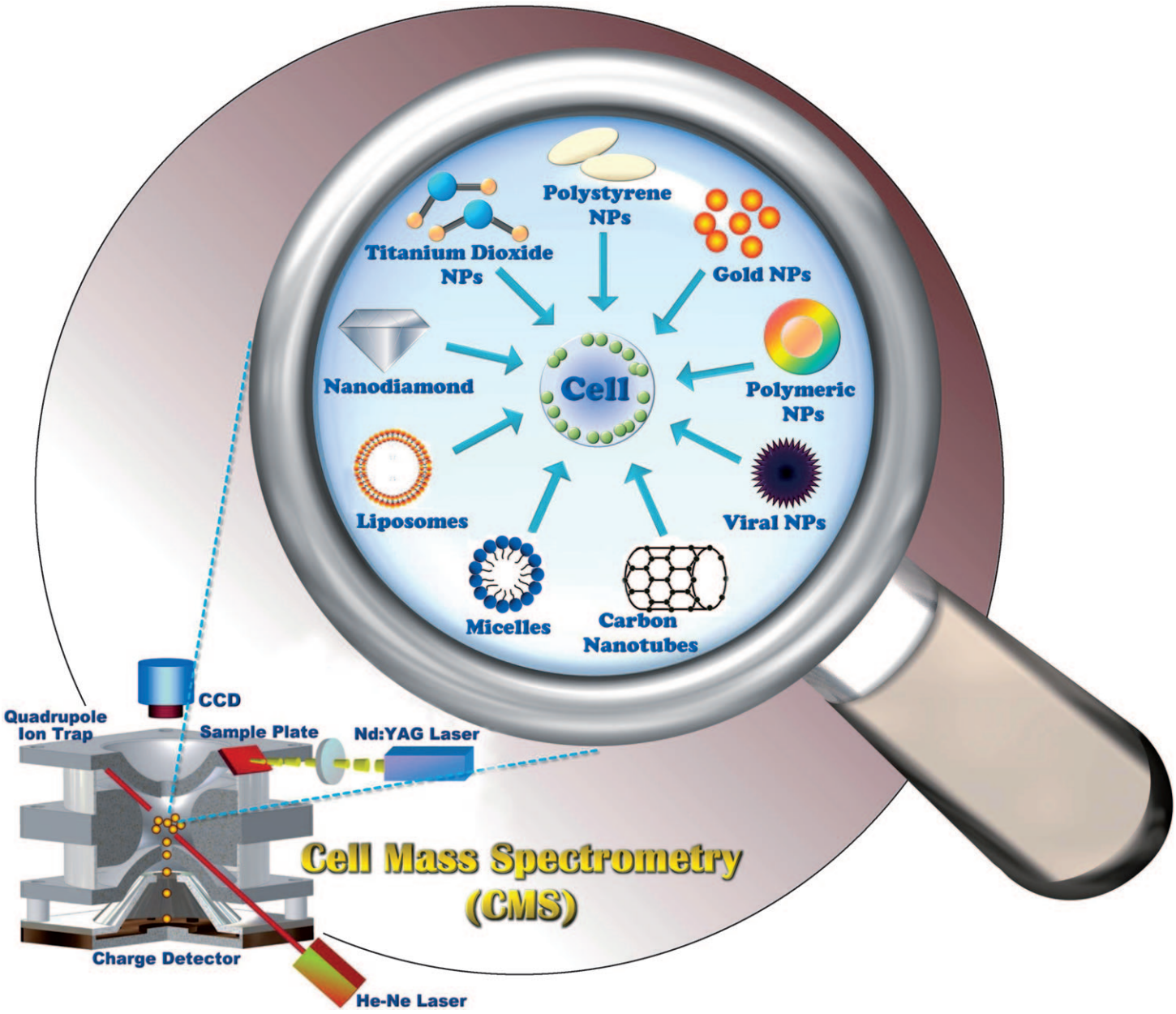

Nano-/microparticles have been pursued as efficient carriers for drug delivery. Therefore, quantitative measurement of the cellular uptake of nano-/microparticles is of great importance for the elucidation of the mechanisms of cell endocytosis and exocytosis.[1–5] One of the motivations for carrying out such measurements is that they may serve as a rapid means to quantify nano-/microparticle uptake into mammalian cells. Conventional methods involve inductively coupled plasma atomic emission spectroscopy (ICP-AES)[1,6] and inductively coupled plasma mass spectrometry (ICP-MS),[7] which are accurate but are limited to elemental species, such as gold nanoparticles (NPs). Recently, laser desorption/ionization mass spectrometry (LDI-MS) was employed for the quantitative measurement of gold-NP uptake by cells, whereby the gold NPs were encoded with different functional groups as "mass barcodes".[8] However, the encoding process is tedious and time-consuming. Furthermore, the ionization efficiencies for various functional groups with different sizes of gold nanoparticles are different, which makes quantitative measurement somewhat uncertain.

In previous experiments,[9,10] we demonstrated rapid determination of the mass distribution of cells and polystyrene microparticles by measuring both mass-to-charge ratios (m/z) and charge (z) simultaneously. Mass spectra over the range of m/z values from 10^9 to 10^{12} were obtained with good signal-to-noise ratios. Different types of mononuclear cells, cancer cells, and red blood cells were clearly distinguished.[9,11,12] A mass resolution of approximately 100 and a mass accuracy of about 1% were also achieved.[12]

Herein, we present a novel approach for investigating the amount of nano-/microparticle uptake into mammalian cells. We developed cell mass spectrometry (CMS)[9–12] to directly measure the masses of cells in the gas phase and found that the quantity of gold-NP uptake into cells as measured by CMS was about the same as that measured by ICP-MS.

To verify that the CMS instrument was able to quantify the cellular uptake of nano-/microparticles, we first incubated NTERA2 cells with gold-NPs with diameters of 30 and 250 nm and recorded their mass spectra. The m/z range of the mass spectra was from 10^9 to 10^{10}. The charges carried on each cell, typically larger than 10000, were detected by a Faraday plate charge detector. The observed mass peaks of NTERA2 cells that had taken up gold NPs were shifted to higher m/z values (Figure 1). Similar experimental results were obtained upon the uptake of 60 nm, 100 nm, and 1 μm NIST polystyrene particles by Raw264.7 cells (Figure 2).[14] The observed mass peaks were in the range of 10^9–10^{10}.

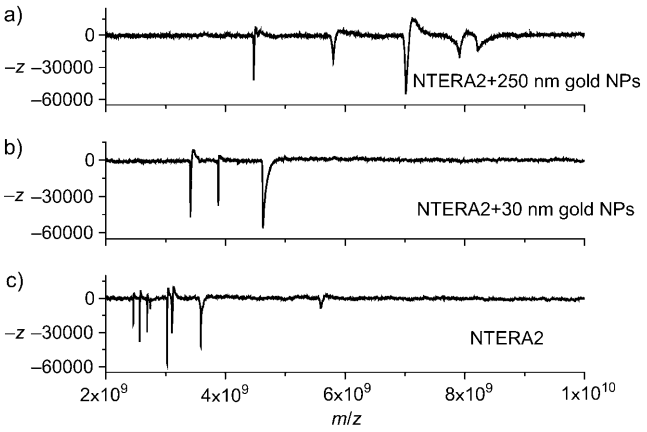

Figure 1. a,b) Mass spectra of NTERA2 cells after the uptake of 250 nm gold nanoparticles (a) and 30 nm gold nanoparticles (b). c) Mass spectrum of NTERA2 cells.

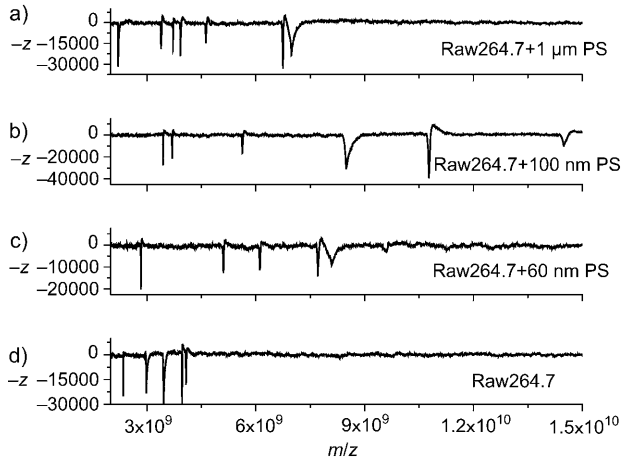

Figure 2. a–c) Mass spectra of Raw264.7 cells after the uptake of NIST 1 μm polystyrene (PS) particles (a), NIST 100 nm polystyrene particles (b), and NIST 60 nm polystyrene particles (c). d) Mass spectrum of Raw264.7 cells.

For mass histogram analysis of NTERA2 cells without gold NPs and NTERA2 cells that had taken up gold NPs (Figure 3), more than 600 cells were subjected to CMS in each case. The acquired m/z values and the corresponding charges (z) on each cell were converted into the absolute mass of the cells studied. The truncation of high-mass clusters was essential during the Gaussian fitting procedure. Mass histogram analysis of the collected mass spectra of 3 μm NIST

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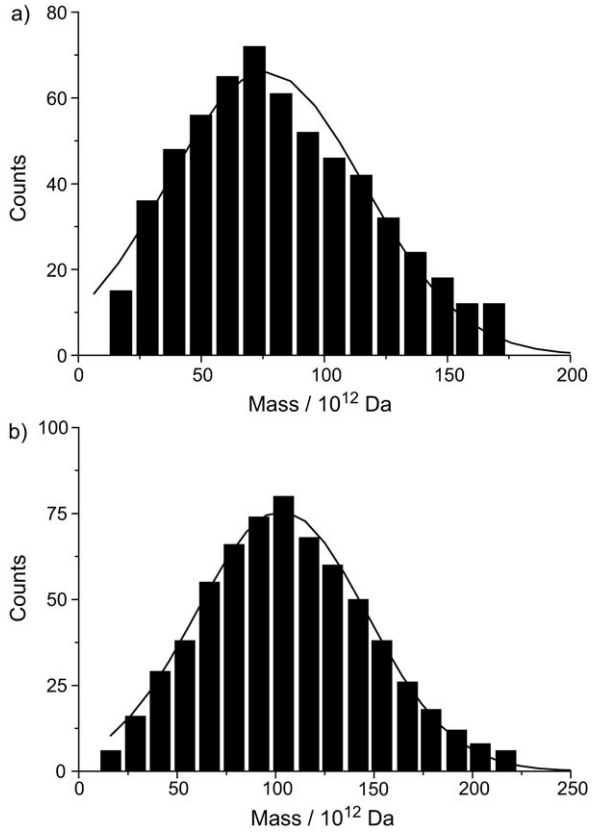

Figure 3. Mass histogram analysis of a) NTERA2 cells and b) NTERA2 cells after the uptake of 30 nm gold nanoparticles.

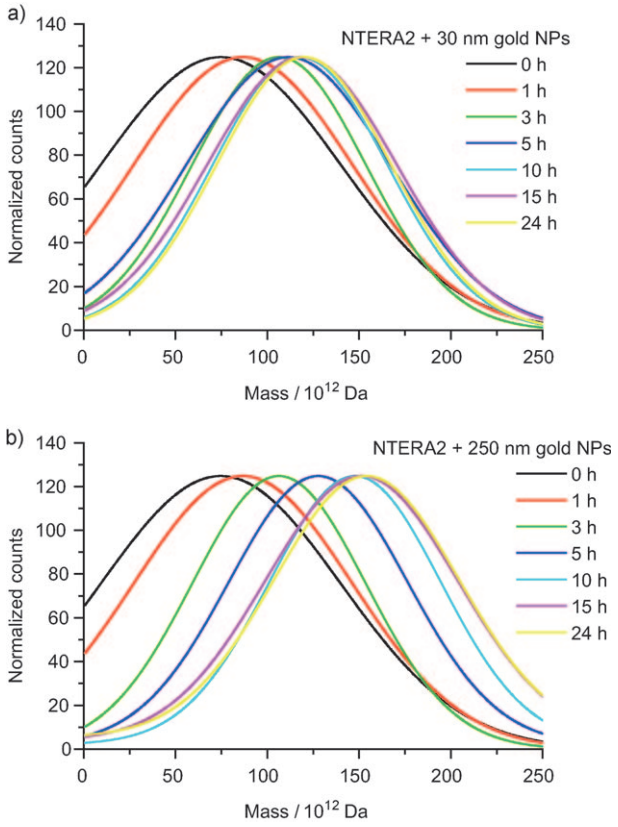

Figure 4. Kinetics of the cellular uptake of a) 30 nm and b) 250 nm gold nanoparticles.

polystyrene particles showed that the mean mass deviated about 1.8% after eight repeated measurements. A mass accuracy of approximately 2.1% was observed for HeLa cells that had taken up gold NPs. Together, these results led us to conclude that the mass accuracy of this CMS instrument is sufficient that it is possible to distinguish between cells with and without gold NPs.

The cellular uptake of gold NPs as a function of incubation time for the NP sizes 30 and 250 nm is shown in Figure 4. The difference in the mean mass was measured to be about 60% after the incubation of NTERA2 cells for 24 h with 30 nm gold NPs. This mean-mass difference corresponds to approximately 260000 30 nm gold NPs (Figure 4a). Figure 4b shows a mean-mass deviation of 200%, which corresponds to an uptake of approximately 800 250 nm gold NPs by the NTERA2 cells after incubation for 24 h.

We determined the kinetics of the uptake of 50 nm gold NPs into NTERA2 cells by both CMS and ICP-MS (Figure 5a). The two methods identified a similar uptake trend and amount. For the CMS measurements, the cell weights were determined after the cells had been fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS). Therefore, CMS measures each individual cell and thus reflects the uptake amounts of gold NPs by cells. These results indicate that cell mass spectrometry is an adequate tool for time-resolved measurements of nanogold uptake by cells. We also measured the cellular-uptake kinetics of polystyrene nanoparticles with the Raw264.7 cell line (Figure 5b). As well as for the quantitative measurement of gold NPs, CMS could be a valuable tool for the quantification all types of nanoparticles,[15] including polymeric NPs,[16] liposomes, viral-based NPs, carbon nanotubes, diamond NPs,[17] and polymeric micelles.

In conclusion, CMS is a rapid and accurate method for determining the quantity of gold-NP uptake into cells. It can be used to determine the number of NPs taken up into each individual cell, whereas ICP provides only a mean uptake for all cells. Furthermore, CMS can be used to measure the cellular uptake not only of metal nanoparticles but also of nonmetal nano-/microparticles.[4] CMS may also be useful for the measurement of nanocarrier uptake, for example, the quantity of liposomes taken up by cancer cells,[18] in drug delivery.[19–21] We plan to apply this technique to evaluate the cellular uptake of viral nanoparticles.[22–24]

Experimental Section

CMS instrumentation and sample preparation: An aliquot (10 μL) of the purified particle suspension (containing approximately 1×10^7 particles/mL) was deposited on an approximately 400 μm thick Si wafer and dried in a desiccator. The experimental setup of CMS comprised laser-induced acoustic desorption of microparticles without a matrix, a low-frequency quadrupole ion trap for the measurement of ultralarge m/z values, a pressure-controlled corona discharge to enhance the number of charges on a cell or microparticle, and a compact, low-noise charge detector for total-charge measurement. A frequency-doubled Nd:YAG (neodymium-doped yttrium aluminium

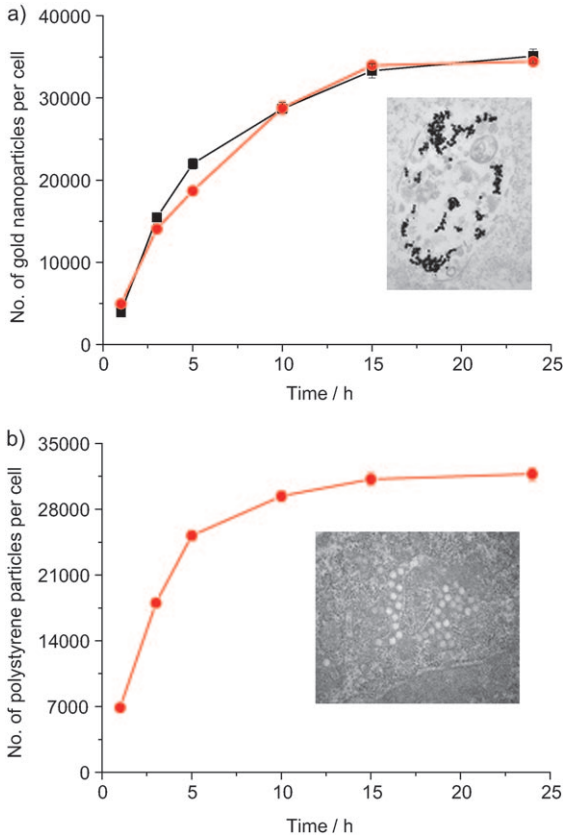

Figure 5. a) Extent of the cellular uptake of 50 nm gold nanoparticles by NTERA2 cells as a function of incubation time, as determined by CMS (red circles) and ICP-MS (black squares). b) Extent of the cellular uptake of 100 nm polystyrene particles by Raw264.7 cells as a function of incubation time. (Inserted photos are TEM images.)

garnet) laser beam ($\lambda = 532$ nm, Laser Technik, Berlin, Germany) with a pulse duration of approximately 6 ns was shone directly onto the back side of the sample plate to desorb cells by laser-induced acoustic desorption (LIAD) with a power density of around 10^8 W cm^{-2}. The laser energy at 30 mJ/pulse was used to irradiate the sample from the back side of the wafer. The radio-frequency (RF) voltage was set at approximately 3000 Vp-p (peak-to-peak voltage). The microparticles released by laser desorption were trapped with an alternating-current (ac) field (ca. 20–350 Hz, 5 s) in a helium buffer gas (ca. 8.7 Pa).

Cell culture: The mouse leukemic monocyte macrophage cell line Raw264.7, the human embryonic-carcinoma cell line NTERA2, and the human cervical-cancer cell line HeLa were obtained from the American Type Culture Collection (ATCC) and cultured according to their specifications with minor modifications.[13] Briefly, cells were cultured at 37 °C under a 5% CO_2 atmosphere in the Dulbecco modified Eagle medium (DMEM) or RPMI 1640 medium containing 10% fetal calf serum, L-glutamine (2 mM), and 1% standard penicillin/streptomycin (10000 IU mL^{-1}; all from Invitrogen, Carlsbad, CA). HeLa, Raw264.7, and NTERA2 cells were incubated with gold nanoparticles or polystyrene particles of various sizes for the indicated times in complete medium. After the allotted time, the cells were detached from the surface of the Petri dish by using the enzyme trypsin, and the cells were homogenized, washed with Dulbecco PBS (Gibco BRL), and fixed with 4% paraformadehyde in PBS for 15 min at room temperature. The cells were then washed three times in distilled deionized water and were counted by using a Vi-CELL series cell-viability analyzer (Beckman Coulter, USA) to determine the total cell number. The cells were resuspended before they were placed in the mass spectrometer or ICP-MS instrument for analysis.

Cell TEM: The cells were first washed thoroughly with PBS buffer. They were then trypsinized and centrifuged at 300 *g* for 5 min, and the supernatant was removed. The cell pellets were fixed with PBS solution. They were then rinsed with cacodylate buffer, postfixed in 1% osmium tetroxide solution, rinsed with 0.1 M cacodylate buffer, dehydrated in ethanol, and rinsed with acetone. The resin was polymerized at 70 °C for 48 h. Ultrathin sections (60–90 nm) were then stained with 5% aqueous uranyl acetate and 2% aqueous lead citrate and imaged with a 75 kV Hitachi H-7000 TEM instrument.

ICP-MS instrumentation: An X series II ICP mass spectrometer (Thermo Electron, Winsford, Cheshire, UK) was used for the determination of gold concentration. A closed-vessel microwave digestion system (MARSXpress, CEM Corporation, USA) equipped with temperature and pressure sensors was used for cell-sample digestion.

ICP-MS sample preparation and measurement: The sample solutions (200 μL) were diluted to 50 mL with deionized water, without a drying step. Cellular-uptake experiments with each AuNP were carried out three times, and the mass spectrum of the product solution of each replicate was recorded seven times by ICP-MS. A calibration standard solution (1000 mg L^{-1}, gold ICP standard, Merck, Darmstadt, Germany) was diluted with deionized water (to 50, 25, 10, 5, 1 ppb). Each standard solution was subjected to ICP-MS, and linear regression was carried out until $\chi = 0.9999$. The resulting calibration line was used to determine the amount of gold taken up by the cells in each sample. A 7% solution of nitric acid was used to wash the instrument between analyses to facilitate gold removal.

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