Joint forces of direct, single particle, CE- and HPLCinductively coupled plasma mass spectrometry techniques for examination of gold nanoparticles accumulation, distribution and changes inside human cells

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Electronic Supplementary Information

Materials used for cell culture

MCF-7 (human breast adenocarcinoma) and MCF-10a (non-tumorigenic breast epithelial) cell lines were obtained from Sigma-Aldrich. For making up the cell cultures the following reagents and materials were used: Dulbecco's Modified Eagle's Medium (DMEM) with and without phenol red (Sigma-Aldrich and Life Technologies, respectively), DMEM/Ham's Nutrient Mixture F-12 with phenol red (Sigma-Aldrich, 51448C), horse serum (Sigma-Aldrich, H1270), fetal bovine serum (FBS, Life Technologies), L-glutamine (200 mM, Sigma-Aldrich), penicillin-streptomycin (10 000 units penicillin, 10 mg streptomycin mL⁻¹, Sigma-Aldrich), epidermal growth factor (Sigma-Aldrich, E9644), hydrocortisone (Sigma-Aldrich, H6909), human insulin (Sigma-Aldrich, I9278), phosphate buffered saline (PBS, Sigma-Aldrich), Tryple Express (Life Technologies), MTT - tetrazolium salt (3-(4,5dimethyl-thiazol-2-yl)-2,5-diphenyltetrazole bromide (Sigma-Aldrich), trypan blue solution (0.4%, Sigma-Aldrich), fast read disposable counting slides (Immune Systems), serological pipettes (Nest Scientific), 75 cm³ tissue culture flasks for adherent cells (Sarstedt), 60×15 mm culture dishes (Sarstedt), 15 and 50 mL falcon vials (Nest Scientific). The equipment used was as follows: a laminar flow cabinet Lamil 10 (Karstulan Metalli Oy), an incubator HERAcell 150 (KENDROMED), an OLYMPUS CKX41 optical microscope, a Hettich Zentrifugen Universal 320R centrifuge, a multi-mode microplate reader Cytation 3 (BioTek).

The cells were cultured in the culture flasks in the incubator (37 °C, 5% CO₂). The MCF-7 cells were grown in DMEM with phenol red supplemented with 10% FBS, 1% penicillin-streptomycin, and 1% L-glutamine. The MCF-10A cells were grown in DMEM/Ham's Nutrient Mixture F-12 with phenol red supplemented with 5% horse serum, 1% penicillin-streptomycin, 10 ng mL⁻¹ epidermal growth factor, 0.5 mg mL⁻¹ hydrocortisone, and 10 μ g mL⁻¹ human insulin. The cells were passaged three times a week.

Uptake studies

The cells were counted using fast read disposable counting slides, seeded on the culture dishes with a density 6×10^5 per dish and incubated (37 °C, 5% CO₂) overnight to be attached to the surface of the dish. Subsequently, the medium was removed and replaced with 3 mL of a solution of AuNPs with different size (5, 10, 20, or 50 nm) containing 19 mg Au mL⁻¹ (the blank test contained no Au) and preincubated in 1000-fold diluted human serum for 45 min. Subsequently, the cells loaded with serum protein conjugated AuNPs were incubated for 45 min, 2, 6, 15, and 24 h. The control sample was a cell culture without incubation with nanomaterial (0 h). One mL of PBS was added to each dish to wash the cells and the washing solution was placed to another vial. Tryple Express (1 mL) was added to each culture dish and incubated (37 °C, 5% CO₂) with cells until they detached from the surface. The cell suspensions were collected to the same vials as previously used. Additionally, 1 mL of PBS was added to each dish to rinse culture dish and transferred to the same vial as used previously. In this way each sample had the same volume (3 mL). The samples were centrifuged (at 1500 rpm for 5 min) and the supernatants were collected to other vials. The cells pellets were suspended in 1 mL of serum-free culture medium and cells were counted using Trypan Blue staining and counting slides. Additionally, 2 mL of culture medium was added to each vial and the samples were prepared for the analysis by ICP-MS.



Fig. S1. Microscopy images of MCF-7 cell cultures incubated with AuNPs of different sizes after suspension in MES buffer and staining with propidium iodide; all the cells (A), cells with damaged membrane (B), A and B pictures combined (C).



Fig. S2. CE-ICP-MS electropherograms of cytosolic fraction after different times of incubation of MCF-7 cells with 20 nm AuNPs preincubated with human serum. 1 - holo-transferrin conjugate; 2 - apo-transferrin conjugate; 3 - albumin conjugate.



Fig. S3. Au signals registered for ionic standard at concentration 1 ppb (A) and for sample of cytosol derived from MCF-7 cells incubated with 50 nm AuNPs conjugated with serum proteins in single-particle mode (B).

Optimization of HPLC-ICP-MS method for the separation of Au species

During the optimization tests different volumes of sample injection were tested (100 and 20 μ L). In order to obtain the lowest peak width that would simplify the separation of Au species, sample volume of 20 μ L was chosen. In the next step the flow rate was optimized (0.2-0.7 mL min⁻¹) to achieve the separation of ionic form of gold from nanoparticles. Flow rate 0.2 mL min⁻¹ provides the best conditions for that purpose. Also, the influence of the concentration of sodium dodecyl sulfate (SDS) in the mobile phase on the separation process was assessed (in the range of 5–10 mM). It was concluded that changing the surfactant content does not affect the migration times and peak areas, so the higher concentration was chosen to help preventing the NPs adsorption on the column packing material.