

Supplementary Material (ESI) for The Analyst
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Efficient intracellular delivery and improved biocompatibility of colloidal silver nanoparticles towards intracellular SERS immuno-sensing

Supplementary Information

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Synthesis and TATHA2-functionalization of AgNPs

Colloidal AgNPs were synthesized using the conventional citrate reduction method reported by Lei and Meisel¹ with some modifications to mitigate AgNPs-specific toxicity by controlling leaching of AgNPs to Ag⁺ ions.^{2,3} One ml sodium citrate (1%) was added rapidly to the boiling solution of 50 ml silver nitrate (0.018%) under stirring. The solution was cooled to room temperature (RT) after reaching a characteristic yellow-greenish color (~ 30-40 minutes). The parameters affecting the transformation of AgNPs by oxidation and dissolution were controlled at the times of synthesis, storage and incubation with cells to improve their biocompatibility. Ultrapure, deionized, molecular biology grade water was used for preparing all solutions. Anaerobic and basic conditions were maintained to avoid oxidation and dissolution of AgNPs to Ag⁺. Exposure of AgNPs to light, cold temperature and centrifugation was minimized.

The protocol for TATHA2 functionalization on AgNPs was adapted from a published method.⁴ The ethyl dimethylaminopropyl carbodiimide/ N-hydroxysuccinimide (EDC/NHS) activated mercapto-methyl-thiazoleacetic acid (MMT) linker (10 µl, 10 mM) was mixed with anti-biotin monoclonal antibody (MAb) (10 µg) for 1 hour at RT. The MMT-MAb solution was filtered through a 1 kDa MW centrifuge filter and the conjugate was incubated with 1 ml AgNPs (70 ppm, roughly equiv. to 7x10¹⁰ NPs/ml) for 1 hour. 1 kDa mPEG-SH (100 µl, 10 µM) as primary blocking agent, followed by biotin-TATHA2 (10 µg) and finally the secondary blocking agent, globulin free and endotoxin low form of BSA (100 µl, 1% BSA), were sequentially added with an incubation time of 20 minutes after each addition. The solution was centrifuged at 2500g for 20 minutes to get a TATHA2-functionalized-AgNPs pellet. To quantify the number of TATHA2 molecules conjugated per AgNP, FITC labeled MAb (Jackson ImmunoResearch, U.S.A.) was used (MAb:TATHA2 is 1:1).

Characterization of AgNPs

The AgNPs were characterized for size, shape, distribution, surface charge, concentration, chemical signature and conjugation using state-of-art techniques: UV-Vis spectrophotometer (Varian Cary, Agilent, U.S.A.), DLS with zeta analyzer (Zetasizer nano-ZS, Malvern, U.K.), TEM (CM200, Philips, Netherlands), ICPMS (ELAN DRC-II, Perkin Elmer Sciex, U.S.A.) and Raman spectro-microscope (RamanStation 400F with RamanMicro

300, Perkin Elmer, U.S.A.). CHNPs, negative control, were synthesized by conventional ionotropic gelation technique by using sodium tripolyphosphate (TPP) as a counterion.⁵ The CHNPs had wide size distribution (40-100 nm), as depicted by DLS.

Evaluation of delivery strategies

To test biocompatibility and uptake efficiency by the delivery strategies, AgNPs were incubated with yeast cells in minimal media for 3, 6 and 12 hours.

Biocompatibility testing: The effect of AgNPs on yeast was observed by cell viability (agar plating) and growth inhibition (optical density/OD at 600 nm and trypan blue dye exclusion) assays in a dose and time dependent study: 1, 10 and 100 ppm for 3, 6, and 12 hours. The silver ions (Ag^+), suspending solution and biotin-TATHA2 were included as test agents in the biocompatibility testing. Chitosan NPs, roughly similar size to the AgNPs (~60 nm) were considered as a negative control. We used an electroporation apparatus and the cuvettes (MicroPulser unit and cuvette with 0.2 and 0.4 cm gap, BIO RAD, U.S.A.) specially designed for cultures in suspension, including yeast. We tested almost every possible parameter (electric field strength, pulse duration and pattern, temperature and electroporation media) to identify optimum electroporation settings to deliver AgNPs into yeast without inducing cellular damage and toxicity, which was validated by SEM (6330F, Jeol, U.S.A.) and agar plating. Electroporation was found to be toxic and was therefore not considered further for uptake studies.

Uptake studies: The number of AgNPs and their localization after cellular delivery via passive and TATHA2 facilitated diffusion was estimated by ICPMS (quantitative uptake) and *in situ* TEM (qualitative uptake). The yeast cells were lysed (Y-PER, Thermo Scientific, U.S.A.) to release the AgNPs, which were then dissolved by acid digestion to Ag^+ , and measured using ICPMS. The cells were sectioned (ultrathin, < 100 nm) by an ultramicrotome (Porter-Blum MT-1, DuPont-Sorvall, U.S.A.) using a diamond knife (DDK, U.S.A.) to observe the intracellular distribution of AgNPs using TEM. The intracellular AgNPs content was quantified using ICPMS, after selective removal of AgNPs from cell-surfaces using a mild I_2/KI etching procedure (0.34/2 mM for 5 minutes),⁶ as validated by SEM.

Delivery technique	Silver nanoparticles (AgNPs)		Cell toxicity	Cell uptake
	Average size (nm)	Average charge (mV)		
Active electroporation	60 ± 8	-40 ± 4	****	N/A
Passive diffusion	60 ± 8	-40 ± 4	*	*, Slow
Facilitated diffusion	85 ± 13	5 ± 7	*	****, Rapid

Table S1. Physicochemical properties of the silver nanoparticles (AgNPs) delivered by three strategies and their corresponding cell toxicity and uptake

Acronyms nm: nanometer, mV: millivolts, number of asterisks represent the relative increase in cell toxicity and uptake of AgNPs. N/A means not applicable, the electroporation was found very toxic and hence not considered for uptake study

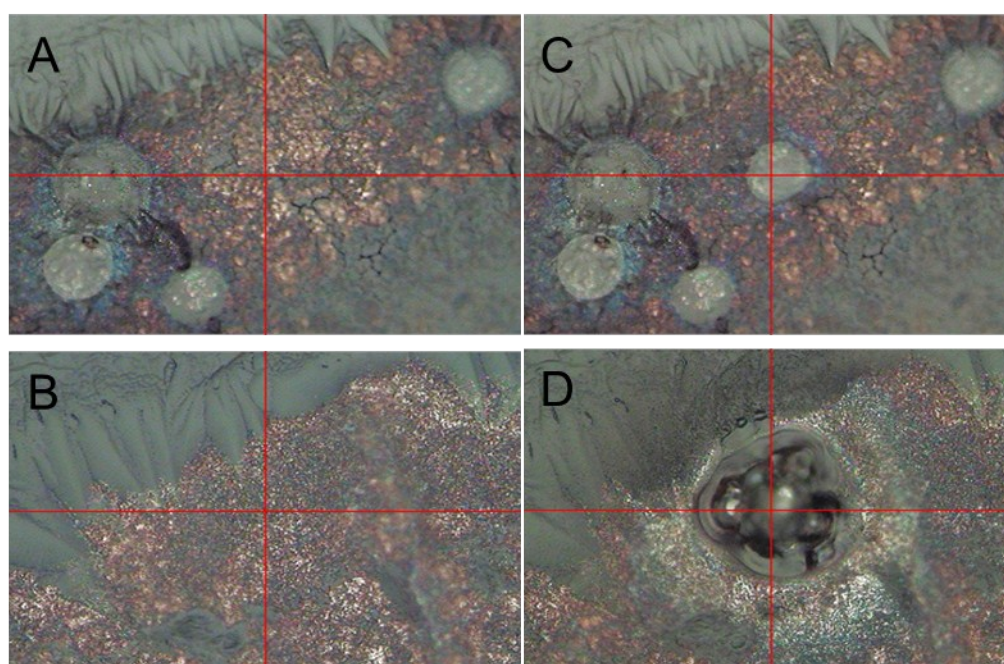


Figure S1. Images of the 10x concentrated AgNP colloids mixed with cell media, before (A and B) and after (C and D) exposure to Raman laser (power 100 mW, acquisition time 5 seconds). The high power Raman laser has damaging effect in presence of AgNPs.⁷ The intersecting lines show the point of laser-focus under Raman microscope.

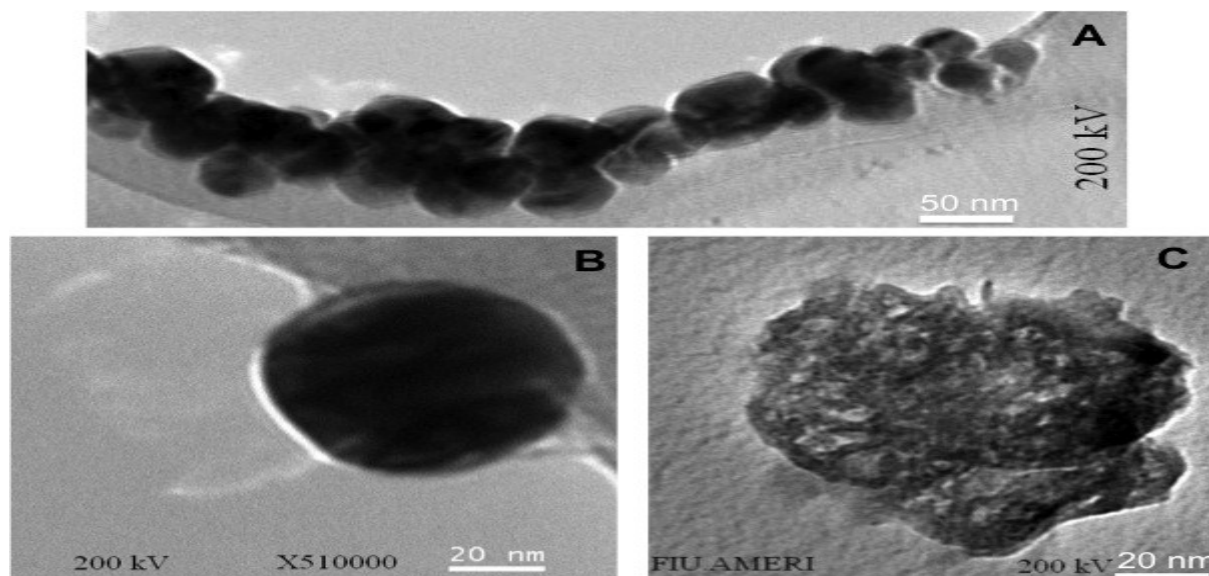


Figure S2. Images of AgNPs showing vaporization (A and B) and shape change or melting (C) under high power TEM (Power 200 kV), consistent with other report.⁸

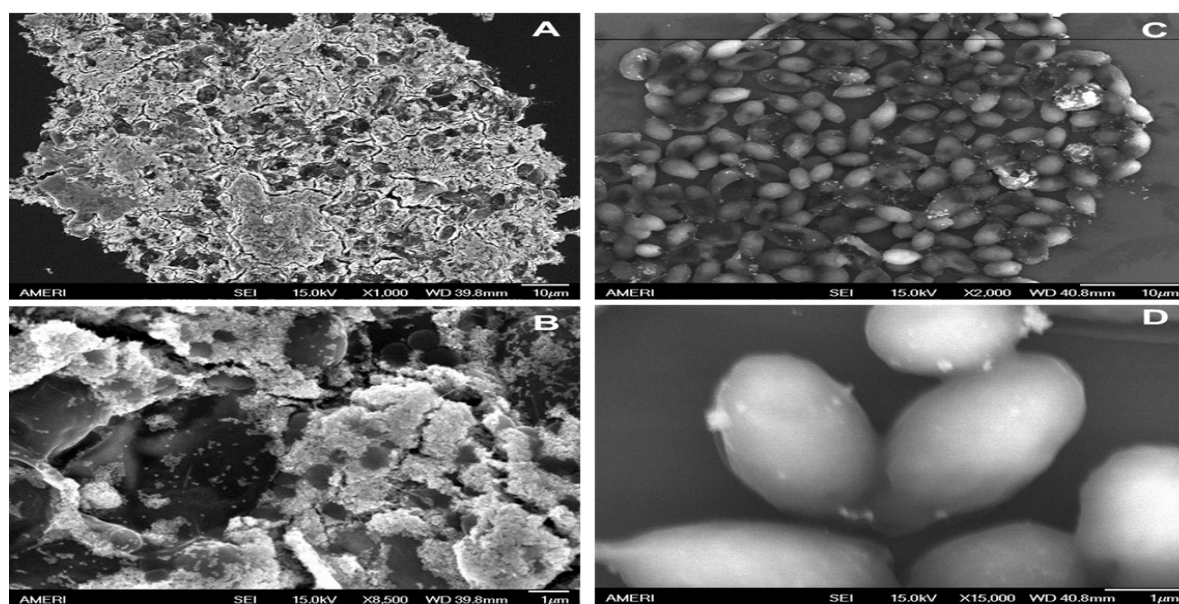


Figure S3. SEM image of cells before (A and B) and after (C and D) I₂/KI etching.

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