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Nanoprobe implantation into mammalian cells by cationic transfection†

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Submicron-sized Au particles and Au/SiO_2 nanocomposites (superparticles) as large as 670 nm have been introduced into tsA201 cells with minimal cell trauma by cationic transfection systems. Successful implantations can be characterized by the expression of co-transfected DNA.

Metal and inorganic nanomaterials are being used with increasing frequency to probe biological systems.[1] Such nanomaterials have applications in drug and gene delivery[2] and biomolecular sensing,[3] and can be used as contrast agents for biomedical imaging.[4] As part of our efforts to design nanoprobes as sensors for intracellular chemical transport, we recently developed a method for assembling colloidal gold nanoparticles into densely packed shells around submicron-sized silica cores (see Fig. 1).[5] These 'superparticle' ensembles respond strongly to visible and near-infrared light, and can serve as substrates for plasmon-enhanced spectroscopies such as surface-enhanced Raman scattering (SERS).[6]

We have investigated several mechanisms for implanting submicron-sized superparticles into live mammalian cells. Previously reported methods for the intracellular delivery of nanoparticles include receptor-mediated endocytosis,[4,7–9] phagocytosis by hepatic Kupffer cells and macrophages,[10] pressurized transfer of nanoparticle suspensions using "gene gun" or direct injection methods,[11,12] acoustically mediated transfer using ultrasound,[3] optically driven implantation using laser trapping,[13] and non-viral delivery systems based on cationic liposomes or surfactants.[14] However, identifying an appropriate method for the efficient implantation of submicron-sized particles proved to be non-trivial. For example, the efficiency of receptor-mediated endocytosis decreases greatly for particles with sizes above 100 nm,[9,15] whereas pressure-driven implantation requires forcing conditions, often resulting in cell injury or death.

Here, we show that the intracellular delivery of nanoprobes can be reliably mediated by cationic liposomal transfection agents. This delivery mechanism is mild and should serve as a general method for the intracellular delivery of nano- and submicron-sized objects.

Colloidal gold nanoparticles (100–250 nm; Ted Pella) and Au/SiO_2 superparticles prepared by electrostatic self-assembly[5] (40/390, 40/550, and 60/550 nm) were employed as nanoprobes. Cationic transfection was evaluated on tsA201 cells using three different systems (TransIT®-293, GenePORTER™, and GeneJuice®). In a typical experiment, a suspension of nanoprobes (10^6–10^7 particles dispersed in 20 μL) was diluted with serum-free medium, then combined with a transfection reagent solution (for full details, see ESI†). Plated cells were incubated with the reagent–nanoprobe mixture in a serum-free medium for 4 h at 37 °C, then washed and incubated for another 24 h in a growth medium containing 1% fetal bovine serum prior to analysis by optical microscopy. For comparison, nanoprobes were also delivered by low power ultrasonication (for full details, see ESI†).

Both cationic transfection and ultrasonication appear to be viable methods for introducing nanoprobes into cells.[16] Superparticles as large as 60/550 nm (diameter 670 nm) can be implanted without apparent injury to the host cell. Individual nanoprobes can be readily detected *via* the intense light scattering produced by excitation of their surface plasmons [see Fig. 2(a)].[17] Particles which exhibited rapid librations or were localized at depths above the plated cells were clearly not internalized and were removed by successive washings. However, nanoparticles adhered on top of cell membranes were more difficult to differentiate from implanted nanoprobes by direct visualization.

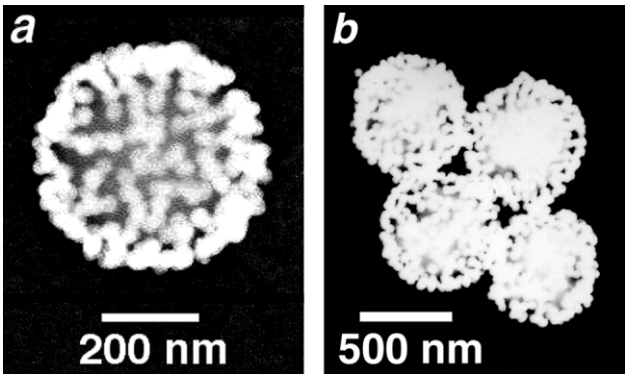

Fig. 1 TEM images (Philips EM-400, 100 kV) of Au/SiO_2 superparticle ensembles. Diameters (in nm): (a) 30/430; (b) 60/550.

To ascertain whether nanoprobes were localized inside cells, a negative stain was applied to cell cultures 24 h after particle delivery.[18] Cells were washed with phosphate-buffered solution (pH 7.2), then immersed in fresh medium containing 1% hydroquinone and exposed to sub-microliter quantities of a 5 mM $AgNO_3$ aqueous solution containing 15% NH_4OH and 5% $(NH_4)_2CO_3$, which was perfused onto cell surfaces using a microinjector (see ESI†). Gold colloid exposed to the staining solution served as nucleation sites for the electroless deposition of Ag, and could be detected within minutes as an amplification of light scattering [see Fig. 2(b) and ESI†]. In contrast, implanted nanoprobes gave rise to negligible enhancement in optical scattering over a period of 10 min, confirming their seclusion from the extracellular environment. Unfortunately, the extracellular particles could not be removed

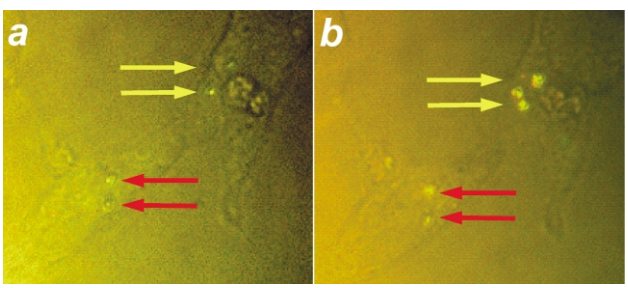

Fig. 2 (a) tsA201 cells implanted with 40/550 nm superparticles after ultrasonication. (b) Cells after addition of $AgNO_3$ (t = 5 min). Scattering intensities from extracellular nanoparticles (yellow arrows) increased rapidly, whereas changes from implanted nanoprobes (red arrows) were negligible during this time period.

† Electronic supplementary information (ESI) available: details of instrumentation, nanoprobe implantation and additional microscopy images. See http://www.rsc.org/suppdata/cc/b3/b317061f/

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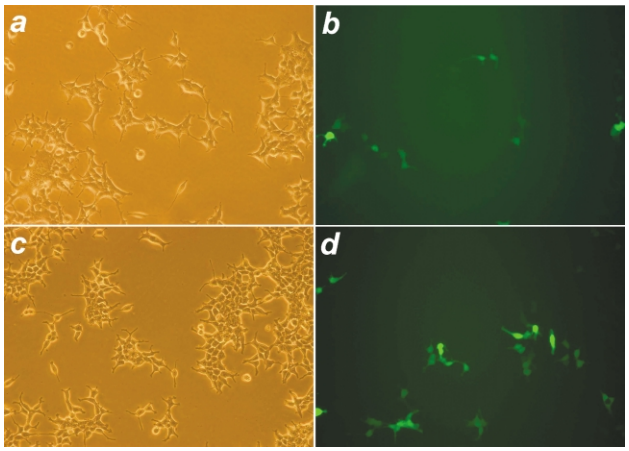

Fig. 3 tsA201 cells implanted, using cationic lipid transfection agents, with 60/550 nm Au/SiO_2 superparticles coated with GFP cDNA: (a, b) TransIT®-293; (c, d) GenePORTER™. The fluorescence images on the right [*i.e.* (b) and (d)] reveal cells expressing GFP 24 h after transfection.

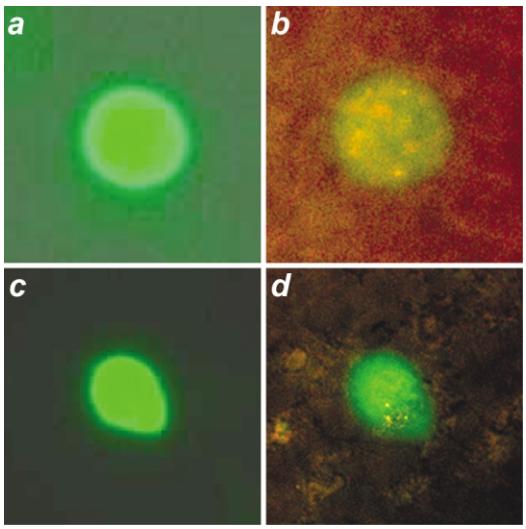

Fig. 4 Second-generation tsA201 cells implanted, using cationic lipid transfection agents, with 60/550 nm Au/SiO_2 superparticles coated with GFP cDNA: (a, b) TransIT®-293; (c, d) GeneJuice®. Cells were imaged in fluorescence mode (left) and with an epipolarization filter (right); the superparticles appear yellow. In the case of image (d), the GFP concentration is sufficiently high that ambient fluorescence is easily detected.

without damaging the cells, rendering light scattering insufficient as proof of nanoprobe implantation.

Cells were also transfected with nanoprobes coated with green fluorescent protein (GFP) cDNA plasmids and evaluated for GFP expression by fluorescence microscopy after a 24 h incubation period (see ESI†). Delivery of DNA-coated nanoprobes by sonication resulted in very little GFP expression, but implantation by cationic transfection resulted in high levels of fluorescence (see Fig. 3).[19] Very importantly, nanoprobes could be detected in more than 90% of the GFP-expressing cells 24 h after transfection; the implantation efficiency ranged from 6 to 10% (see ESI†).[20] Several schemes can be developed for correlating implantation with DNA co-transfection; for example, positive cell selection using plasmids encoded with resistance genes should work equally well. Expression of co-transfected cDNA plasmids can thus serve as a convenient metric for determining successful nanoprobe implantation and establishing cell viability.

Superparticles implanted by cationic transfection do not appear to impair normal cell growth or function. No significant differences in viability were observed upon comparison of cells with and without nanoprobe implants (see ESI†). Furthermore, the nanoprobes can be passed on by mitosis to daughter cells for several generations, confirming their retention within the cells as well as validating their biocompatibility (see Fig. 4 and ESI).[21] Evaluation of these nanoprobes as intracellular sensors of chemical uptake is currently under way.

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