One-pot synthesis of silica-coated magnetic plasmonic tracer nanoparticles†

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We demonstrate a one-pot procedure to synthesize and embed silver nanoparticles inside silica shells together with iron oxide nanoparticles and Raman reporter molecules, followed by fluorophore attachment to the silica, to form a class of tracer nanoparticles suitable for biological and environmental applications.

The areas of bio-sensing and bio-imaging have benefited with the advent of quantum dots (QDs) and their superior spectroscopic properties over conventional organic dyes.[1] Unfortunately, the use of cadmium-based materials for *in vivo* studies could be hindered by potential cytotoxicity.[2] On the other hand, metallic nanoparticles demonstrate exciting plasmonic properties which offer capabilities such as single molecule detection by surface-enhanced Raman scattering (SERS),[3] optical imaging *via* wavelength-tunable intense elastic light scattering without photo-bleaching or blinking,[4] chemical and bio-sensing *via* analyte-induced colorimetric changes,[5] photothermal therapies,[6] and relatively low cytotoxicity (compared to quantum dots).[7] For biological applications, the ideal nanoparticle tracer would combine multiple detection modalities (magnetic, fluorescence, elastic or inelastic light scattering), be stable under biological conditions, offer surfaces that could be easily conjugated to bio-markers and show minimal toxicity. To develop such multi-property nanoparticles, recent studies investigated coupling QDs with superparamagnetic iron oxide nanoparticles (SPIONs),[8] iron oxide-noble metal core-shell nanoparticles[9] and fluorophore-doped reporter particles.[10] The coupling of SPIONs to nanoparticles broadens their application scope for MRI contrast imaging, bio-labelling and bio-separation, coupled with fluorescence (QDs) or plasmonic (metallic) properties.[8–10]

In this Communication, we report a one-pot synthesis of silica-coated tracer nanoparticles with low cytotoxicity that have magnetic, elastic light scattering, and SERS properties. The silica shell on these particles can be easily modified to attach fluorophores or bio-markers. Thus, the particles could be qualitatively tracked in environmental or biological media in time and space using optical techniques, plus be separated from the media by their magnetic properties. The synthesis procedure involves a microemulsion route[11] for the synthesis of silver nanoparticles inside a silica shell in the presence of pre-made iron oxide nanoparticles and a Raman reporter molecule (4-mercaptobenzoic acid, 4-MBA). The details of this procedure are outlined in the electronic supplementary information (ESI).†

TEM images (Fig. 1A, B) for these samples indicate quite uniform silica spheres (size = 45 nm ± 7 nm) with dark particles in the cores. The measured core particle size (d = 21 ± 5 nm) suggests that these dark particles are presumably silver nanoparticles, as the pre-made iron oxide particles are much smaller (8–10 nm).[12] The higher magnification image (Fig. 1B) shows 1–4 silver or iron oxide particles encapsulated inside silica cores. Inductive coupled plasma (ICP) measurements were performed, after acid digestion of the samples, to determine the silver and iron content. Energy dispersive analysis of X-rays (EDAX) was used to determine the Si and Si : Ag atomic percent ratios. Such analysis and subsequent calculations indicates an approximate concentration of one silver

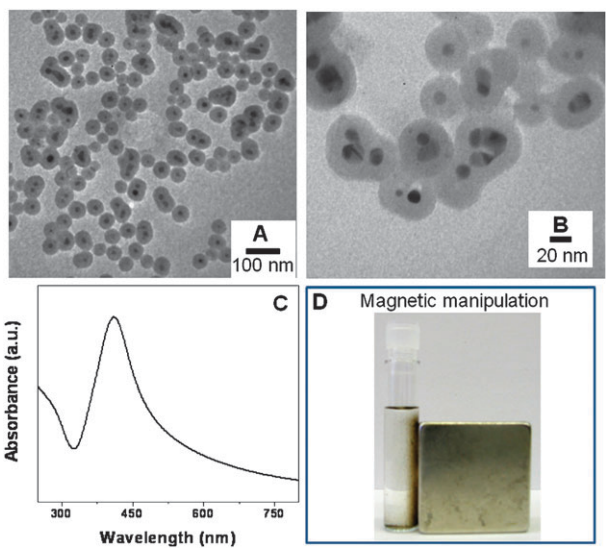

Fig. 1 Low (A) and high (B) magnification images of composite particles consisting of silver and iron oxide nanoparticles embedded in silica particles. (C) UV–Vis spectrum of the composite particles. (D) Photograph of the particles in a glass tube after placement of a magnet for 30 minutes.

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nanoparticle per 3 silica particles and about 3 iron oxide nanoparticles per silica particle respectively (detailed calculations are included in ESI). It is possible that some silica particles do not contain silver nanoparticles and might mainly contain iron oxide particles.

The UV–Vis spectrum of these tracer particles (Fig. 1C) shows a prominent and a broad surface plasmon absorption band centered at 411 nm,[13] indicating the presence of silver nanoparticles. The presence of iron oxide in the particles allows magnetic manipulation of these particles, which can be seen by placing a neodymium iron boride ($Nd_2Fe_{14}B$; $\sim$1 tesla) magnet (Fig. 1D) near the particles. Magnetic hysteresis data obtained by a vibrating sample magnetometer also confirm the presence of iron oxide particles in the samples (data not shown for brevity).

The tracer particles were further studied by darkfield light scattering. A 100 μL tracer particle solution with 10^{-4} M silver atom content was placed on a glass slide and imaged by a Nikon Eclipse model ME600L microscope equipped with bright field, dark field, and fluorescent imaging capabilities. Bright greenish-yellow light is scattered from these particles (Fig. 2A) due to the elastic light scattering of silver nanoparticles.[13] Control experiments with plain silica particles and silica particles embedded with iron oxide particles in the absence of silver nanoparticles showed significantly less scattering (data shown as Fig. S1 and S2 in ESI†).

Silver nanoparticles are good substrates for SERS. As mentioned earlier, a Raman tag (4-MBA) was included along with the microemulsion synthesis of these particles. We believe that the thiol group of 4-MBA attaches to the silver nanoparticle surface. A strong SERS signal is evident (curve 1, Fig. 2B); the two prominent bands at 1075 cm^{-1} and 1582 cm^{-1} correspond to the ring breathing modes of the 4-MBA molecule.[14] For comparison, silica particles with pre-made iron-oxide-4-MBA in the absence of silver nanoparticles (curve 2) and plain 4-MBA molecules dispersed in water (curve 3) were also studied and show far less Raman signal.

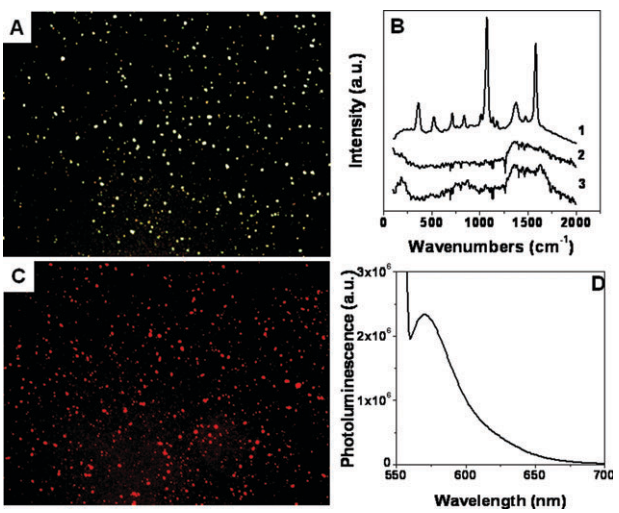

Fig. 2 (A) Elastic light scattering image recorded for the composite particles. (B) SERS spectra for the 4-MBA molecule attached to the composite particles. The vibrational peaks are assigned in the text. (C) Fluorescence image recorded for the composite particles to which NHS-rhodamine is attached. The red fluorescence arises from the rhodamine dye. (D) Fluorescence spectra recorded for the NHS-rhodamine dye attached to the composite particles (Ex = 552 nm).

The presence of the silica surface allows further surface modification of these particles, which is an important step for bioconjugation. To demonstrate this, we incubated 1 mL of 1.5 nM tracer particles with a 10^{-2} M aqueous solution of aminopropylsilane (APS) for 12 h. This leads to amine functionalization of the surface. The zeta potential measurements in water before (-50 mV $\pm$ 0.8 mV) and after ($+16$ mV $\pm$ 1 mV) the reaction support successful surface modification. The light scattering size marginally increases from 183 nm to 222 nm indicating some amount of aggregation due to a reduction of overall charge on the nanoparticle surface. To these particles, 0.5 mg of NHS-rhodamine (Pierce) was added followed by vigorous vortexing. The particles were protected from light to avoid any fluorescence quenching. A fluorescence microscopy image of these particles was recorded (Fig. 2C) with a corresponding fluorescence spectrum (excitation at 552 nm) for these particles (Fig. 2D), qualitatively confirming rhodamine attachment.

To calculate the amount of rhodamine bound to the tracer particles, different concentrations of aqueous NHS-rhodamine solutions were studied by UV–Vis spectroscopy. Using a standard curve thus obtained and using the relation (rhodamine added to the particles) – (rhodamine in supernatant left after centrifugation/purification of particles) = rhodamine bound to the tracer particles, we arrive at a concentration of 4.8×10^{-6} M rhodamine molecules bound per mL of tracer particle solution. Taking into consideration the number of silica particles present (calculated using ICP and EDAX data), the number of rhodamine molecules per silica particle was estimated to be 3200. This number is close to what one would expect for full surface coverage of rhodamine, on individual silica particles. Furthermore, using rhodamine 6G as a standard, the quantum yield (QY) for the same amount (4.8×10^{-6} M) of NHS-rhodamine in solution and that on the surface of the tracer particles was calculated to be $\sim$11.2% and 2.3%, respectively. This indicates some amount of fluorescence quenching, possibly due to the close vicinity of silver nanoparticles. With the fluorescence property now included, the particles are now truly multifunctional tracer particles with magnetic, light scattering, SERS and fluorescence properties.

The cytotoxic behavior of these particles was studied on HT29 human colon adenocarcinoma cells by using the MTT assay, the details of which can be found in the electronic supplementary information (ESI).† Cell viability decreases as the concentration of the tracer particles increases (Fig. 3A). It is important to note here that the concentrations mentioned are those for the silver nanoparticles embedded inside the silica cores of the tracer particles (as determined by ICP). We find roughly a 70% cell viability for 10^{-8} M concentration of silver particles which improves at lower concentrations (80% cell viability for 0.3 nM) (Fig. 3A). The reasons for toxicity of these particles are not clear at this point, although some free silver nanoparticles without silica coatings could contribute to such toxicity. Wang *et al.* observed $\sim$75% cell viability at 1×10^{-11} M concentration of silver nanoparticles.[15] In comparison, we observed higher cell viability $\sim$80% at two orders of

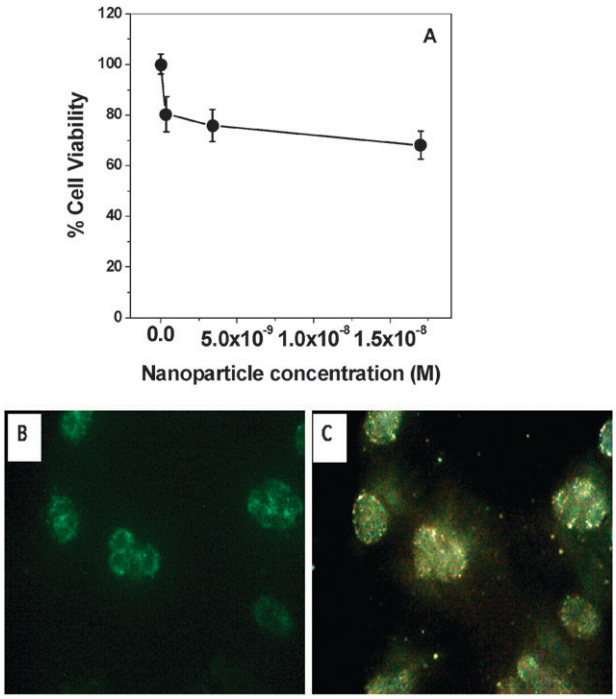

Fig. 3 (A) Cell viability studies for HT29 human colon adenocarcinoma cells as a function of concentration of silver nanoparticle content (molar) in the composite particles. (B) Fluorescence microscopy and (C) darkfield light scattering images of the HT29 cells incubated with the green fluorescent dye calcein along with the tracer nanoparticles for 3 hours.

magnitude higher concentration of silver nanoparticles ($\sim$nM). This makes our tracer nanoparticles less cytotoxic than bare silver particles, yet with retention of the desirable optical properties of silver nanoparticles. To visualise cellular uptake, HT29 cells were plated on a Lab-Tek II chamber slide system at 2.5×10^4 cells per chamber. Calcein (Invitrogen) was mixed with the tracer nanoparticles (25 μL) and added to the cells at a concentration of 100 μg ml^{-1}. Calcein is a polyanionic, membrane impermeable fluorophore, which is taken up by endocytosis/pinocytosis and exhibits a green fluoresence upon excitation at 485 nm (Fig. 3B).These cells were then incubated at 37 °C for 3 hours, washed with PBS and imaged live for darkfield light scattering and fluorescence. The darkfield image (Fig. 3C) shows a bright yellow-green scattering from the tracer particles, which makes these particles excellent candidates for bio-imaging applications. Control experiments without the tracer particles failed to show such brilliant scattering (ESI, Fig. S3†).

In conclusion, we have demonstrated the synthesis of silica coated, silver-containing tracer particles with magnetic, light scattering and SERS properties that show lower cytotoxicity compared to free silver nanoparticles. We further demonstrate facile surface modification by the attachment of rhodamine dyes. These tracer particles can be used for bio-separations, SERS sensing and bio-imaging by fluorescence and darkfield light scattering techniques, thus making them truly multifunctional tracer particles that hold promise for different biomedical applications.

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