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Gold nanoparticles explore cells: Cellular uptake and their use as intracellular probes

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ABSTRACT

Understanding uptake of nanomaterials by cells and their use for intracellular sensing is important for studying their interaction and toxicology as well as for obtaining new biological insight. Here, we investigate cellular uptake and intracellular dynamics of gold nanoparticles and demonstrate their use in reporting chemical information from the endocytotic pathway and cytoplasm. The intracellular gold nanoparticles serve as probes for surface-enhanced Raman spectroscopy (SERS) allowing for biochemical characterisation of their local environment. In particular, in this work we compare intracellular SERS using non-functionalised and functionalised nanoparticles in their ability to segregate different but closely related cell phenotypes. The results indicate that functionalised gold nanoparticles are more efficient in distinguishing between different types of cells. Our studies pave the way for understanding the uptake of gold nanoparticles and their utilisation for SERS to give rise to a greater biochemical understanding in cell-based therapies.

1. Introduction

Stem cells, understanding molecular mechanisms underlying the development of cancer and neurodegenerative diseases as well as studying the interactions of pathogens such as viruses and bacteria with cells are topical areas in molecular biomedical research. Though fluorescence microscopy is a major tool for cellular imaging, it has known limitations such as the invasiveness of the labelling (or staining) process, inability to monitor multiple molecular targets interactions as well as it suffers from photobleaching. Therefore, new imaging techniques and approaches that can identify molecules and interactions at sub-cellular resolution are very much required. One of the ways to achieve these objectives is to combine the use of nanoparticle-probes with intracellular molecular spectroscopy. Gold nanoparticles (AuNPs) can be used as probes and they have recently attracted a lot of attention in biomedical research due to their chemically inert nature and remarkable optical properties. AuNPs have been utilised in many applications such as biochemical sensing [1], drug and gene delivery [2,3]. The rich optical properties of AuNPs arise from their ability to localise surface plasmons. This also enables their use as transducers for surface-enhanced Raman spectroscopy (SERS).

SERS is a highly sensitive, label-free and non-destructive method which allows for molecular identification giving it several advantages over other imaging techniques such as fluorescence, infrared, UV–visible or NMR [4–7]. In SERS, the otherwise extremely weak Raman scattering signal [8] is enhanced by several orders of magnitude by ensuring that the molecule is in very close vicinity of nanoparticles or of a nanostructured gold or silver surface [7,9,10]. The SERS spectrum is a vibrational 'fingerprint' which characterises the chemical bonds and symmetry of the molecule. [4] Recently, nanoparticle-based SERS has been employed in many biomedical applications from chemical sensing [11–13] to cancer detection in body fluids and tissues [14–17].

In contrast to conventional methods stated earlier, higher detection limits in addition to a complete structural characterisation of the target molecule can be achieved with SERS [18] without the need for staining or expressing fluorogenic proteins. It has also been demonstrated that SERS can be applied to living cells in order

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to monitor cellular functions [19], cell response to stress [20] and apoptosis [21]. SERS has been extensively applied in biomedical applications often for label-free detection of biomolecules in cells and tissues [4,22]. AuNPs have been used as intracellular probes for SERS [23] to monitor release of drugs inside cells [24] as well as to probe molecules by targeting them to cellular compartments such as mitochondria, endosomes and the cell nucleus [11,22,25,26]. The key for utilisation of nanoparticles inside cells for SERS and other measurements is their mechanism of uptake. Particle uptake has been achieved using physical methods such as microinjection [27,28], electroporation [29], sonoporation [23,30] and gene gun [31]. Further options for involuntary uptake are the translocation of particles through the plasma membrane facilitated by cell penetrating peptides (CPPs) [32,33] such as the trans-activating transcriptional protein derived from viruses [34].

Moreover, AuNPs are known to be taken up by cells voluntarily whereby their intracellular uptake and distribution depends on many factors such as particle characteristics, e.g. charge, surface modification, particle size and shape [35–39] as well as experimental procedures involving concentration and exposure time [40]. Different cellular mechanisms are involved in particle uptake such as phagocytosis and pinocytosis. The latter facilitates the uptake process through small membrane-bound vesicles, called endosomes.[41,42] Pinocytosis can involve energy-dependent and receptor mediated endocytosis which has been shown to be the dominant internalisation pathway for several cell lines [36,40,43]. Following internalisation, membrane-bound vesicles encapsulating the particles mature and eventually fuse with lysosomes [41].

Hence, intracellular AuNPs are trapped inside membrane-bound vesicles of the endocytotic pathway [37]. Escape from these vesicles can only be achieved by functionalisation of the particle surface with peptides such as CPP [33,34] and adenoviral receptor-mediated endocytosis peptides [44]. Trapped in endocytotic vesicles, particles get transported through cells via the common cellular transport mechanisms: by molecular motors such as myosin, kinesin and dynein along the intracellular filament network [45]. Studies have revealed various characteristics of these motor proteins *in vitro* and *in vivo* with reference to endocytotic organelles being transported. Friedman and Vale measured the *in vivo* mobility of kinesin unattached to a surface using single molecule assays to be 600–800 nm/s [46–48]. Further studies revealed that average velocities strongly depend on the size of the attached cargo that is transported by these motors: a larger size appears to correlate with slower motions [49]. For example, 30 nm quantum-dots tagged to kinesin showed an average velocity of 600 nm/s in HeLa cells [49], whereas 1 μm collagen-coated beads in murine embryonic fibroblasts displayed a velocity of ~10 nm/s [50] and 3 μm polystyrene beads in SV80 human fibroblasts showed similar velocities of 8–30 nm/s [51] suggesting that many factors influence the transport speed in cells. These factors include variations within the cell line, the bead size and its surface properties and material composition, which change the stall force of the motors and steric hindrance within the cell [50]. Moreover, the velocity of endocytotic vesicles such as endosomes in budding yeast and lysosomes in African green monkey kidney cells was tracked and gave values of (213 ± 139) nm/s [52] and ~410–450 nm/s [53], respectively.

In this work, we probe cellular uptake and dynamics of AuNPs optically. The particle uptake is studied with non-functionalised (citrate-capped) AuNPs revealing different trajectories and speeds which correlate with different transport and diffusion mechanisms inside cells. Such internalised AuNPs serve as intracellular SERS probes. We use these nanoparticle-probes taken up through the endocytotic uptake pathway to report SERS and utilise this information to evaluate their ability to segregate different cell phenotypes. Further we also employed AuNPs functionalised with nuclear localisation signal peptide (NLS) as SERS probes. The functionalised nanoparticle-probes give much better cellular phenotype distinction compared to non-functionalised AuNPs. This work reaffirms the nanoparticle-probe based SERS methodology for intracellular investigations and for achieving cellular distinction.

2. Methodology

2.1. Cellular uptake and dynamics of AuNPs inside cells

All experiments were carried out on undifferentiated (UDC) and differentiated (DC) SH-SY5Y cells, a human neuroblastoma cell line, cultured and maintained as described elsewhere [26]. Both cell phenotypes are adherent and display a flat and neuronal morphology. Fluorescence staining with Hoechst 33343 (Invitrogen, UK) and anti-dopamine antibody (Anti-DA) (mouse, 1/1000, Millipore, UK) were carried out according to staining procedures described elsewhere [26,54]. Cells were grown in collagen-coated glass bottom dishes (MatTek, US) and incubated with citrate-capped spherical AuNPs (BBinternational, UK) of different diameters (40, 60 and 100 nm) at a concentration of 200,000 particles per cell independent of particle size illustrated in Fig. 1A. The uptake of particles is shown for an incubation time of 24 and 48 h in Fig. 1B–D for 40, 60 and 100 nm particles. Based on these images, internalisation of particles is visible. We could observe aggregates of nanoparticles inside cells, which appear as dark spots. No aggregation of AuNPs is observed in the cell culture medium (see Supporting Information) and therefore we believe that aggregation is induced inside the endocytotic compartments. There is no indication that non-functionalised, intracellular AuNPs are localised outside the endocytotic pathway as also observed using transmission electron microscopy by Tkachenko et al. [37]. In the images shown in Fig. 1B–D, some nanoparticles can be seen outside cells. These are immobile aggregates which stick to the coating of the cell culture dishes.

In order to track and characterise the motion of particles or intracellular vesicles, they have to be imaged in a time-dependent way that allows extraction of important biophysical parameters like speed of the particle and the diffusion constant. This is commonly performed by measuring the mean-square displacement (MSD) of a particle within a given lag time Δt. It is calculated in two dimensions as follows:

$$\begin{aligned}\mathrm{MSD}(\Delta t) &= <(\Delta r(\Delta t))^2> \\ &= <(x(t)-x(t+\Delta t))^2+(y(t)-y(t+\Delta t))^2>. \end{aligned} \quad (1)$$

The resulting trajectories can be classified into models describing different types of motions such as anomalous subdiffusion or confined random walk

$$\mathrm{MSD}(\Delta t) = 4D\Delta t^{\alpha} \quad (2)$$

and directed motion as superposition of diffusion and transport

$$\mathrm{MSD}(\Delta t) = 4D\Delta t + (v\Delta t)^2. \quad (3)$$

While based on the Einstein-Stokes relation, the diffusion constant D for spherical particles subject to Brownian motion in two dimensions can be described as

$$D = k_B T/(4\pi\eta r) \quad (4)$$

where k_B is the Boltzmann's constant, T is the absolute temperature, r is the particle radius and η is the fluid shear viscosity.

For tracking nanoparticles, bright field images of live cells were obtained in trans-illumination using a 40× condenser (NA = 0.6) and a 100× oil immersion (NA = 1.4) objective with a white light source on a confocal microscope system (Leica, Germany). Images