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Quantifying nanoparticle-cell interactions

Nicole Hondow[1], Andy Brown[1], Huw D. Summers[2], M. Rowan Brown[2], Paul Rees[2], Mark D. Holton[2], and Rik Brydson[1]

[1.] Institute for Materials Research, School of Process, Environmental and Materials Engineering, University of Leeds, Leeds LS2 9JT, UK.
[2.] Centre for Nanohealth, College of Engineering, Swansea University, Swansea SA2 8PP, UK.

Nanoparticles are finding increasing applications in the field of medicine [1], with extensive research being conducted on the design of nanoparticles to enhance targeted uptake and action [2]. Preliminary investigations of nanoparticles with potential applications in medicine involve in vitro testing to explore cellular uptake (particle dose) and action. Analytical transmission electron microscopy (TEM) is a valuable tool for the intracellular localization of nanoparticles and for the development of mechanistic modes of action. TEM studies can be conducted on the nanoparticles prior to in vitro delivery and subsequent to it on ultrathin sections of cells.

We hypothesize that we can use electron microscopy to investigate nanoparticle dose, linking the initial dispersion of nanoparticles in biological media through to the uptake by human osteosarcoma (U-2 OS) cells. Quantitative cell (optical) imaging has been used to show that the nanoparticle uptake into cellular vesicles proceeds by a random process [3]. This was achieved by assuming groups of quantum dots within vesicles act as discrete and stable fluorescent sources. We have prepared standard thin sections of these cells for correlation of TEM with the optical results [4]. TEM imaging can identify and quantify the number of quantum dot loaded vesicles per cell section [4] and the number of dots per vesicle (Figure 1).

Nanoparticles used for in vitro studies such as these quantum dots are often coated with polymers or proteins to facilitate entry into cells. One property that can affect uptake is the agglomeration or dispersion state, and this is often well characterized in simple solutions by light scattering techniques. However, the particle dispersion is less well characterized when the nanoparticles are dispersed in solutions of biological media and serum for delivery to cell lines, due to the interaction with and scattering from the (protein) media. TEM analysis of nanoparticle dispersions in solution has previously been limited, in the main, to the measurement of primary particle size due to the drying effects that occur during basic sample preparation. However, solutions prepared for TEM by plunge freezing specimen grids coated with a blotted solution of nanoparticles is a sensitive and representative route to measuring nanoparticle dispersion, especially as the nanoparticle dispersion remains stable as the grid is warmed to room temperature, allowing for extensive TEM imaging and analysis [5].

Quantitative analysis of the serum-quantum dot solution used in [3] and [4] shows a heterogeneous dispersion of dots with a high frequency of monodisperse dots plus a significant (volume) fraction of agglomerates. Quantitative TEM imaging of cellular uptake shows a similar distribution of uptake in vesicles and we have correlated this distribution to the distribution identified by optical metrology [3]. This has been extended into 3-D, using the Gatan 3-View system in the scanning electron microscope (SEM) to serial section resin-embedded cells exposed to quantum dots. Through combining the information from TEM imaging (i.e. location and number of vesicles per 2-D cell section plus the number of quantum dots per vesicle) and serial sectioning (i.e. location, size and number of vesicles in

whole cells (3-D)) with that provided by optical imaging, we have quantified nanoparticle dose and dose dispersion following of cellular uptake by targeted quantum dots [4].

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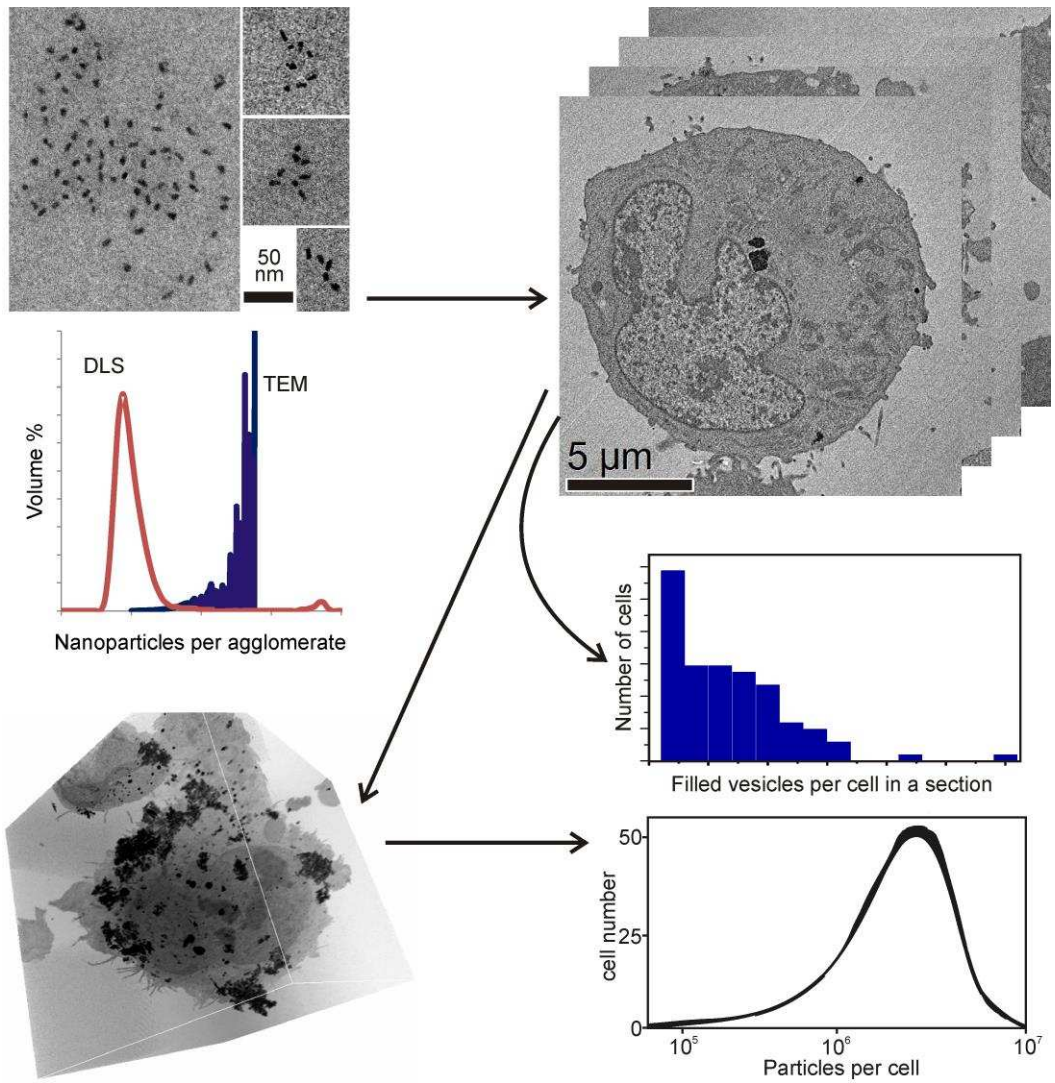

Figure 1. The use of quantitative electron microscopy to study nanoparticle-cell interactions; initial investigation of quantum dot agglomeration state can be linked to the cellular uptake, with TEM imaging providing numbers of filled vesicles and numbers of quantum dots per thin section which is then related to 3-D and flow cytometry.