Multiscale Correlative Microscopy of the Interaction of Au Nanoparticles with Rat Cortex Neural Progenitor Cells

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Nanoparticles are being engineered for biomedical applications, where they may provide imaging contrast, deliver drugs or nucleic acids, or be employed to track implanted stem cell therapies. These applications involve particle internalization. Particles may not be internalized by the desired cells or may translocate to non-targeted regions of the body. In order to effectively design particles, it is important that nanoparticle-cell interactions are characterized at a variety of scales, tracking the nanoparticle in its new biological environment. By correlating images from instruments that have different resolutions and contrast mechanisms, it is possible to determine whether nanoparticles have been internalized by specific, individual cells and where they reside within the cells. Rat cortex neural progenitor cells, which differentiate into the major cell types found in the brain, are a biological model that can be studied at different stages of development[1]. These cells were exposed to citrate-stabilized gold nanoparticles, a versatile system for biomedical applications. Nanoparticle internalization was characterized through the use of optical, electron, and ion imaging tools.

To investigate nanoparticle internalization, neural progenitor cells were obtained commercially and proliferated in suspension culture, forming neurospheres. Single cell suspensions were seeded onto glass coverslips or carbon-coated sapphire discs (1250 cells/mm^2) and allowed to differentiate. All substrates were treated with poly-L-ornithine to promote cell adhesion. Cells on glass coverslips were exposed to 30 nm gold particles (5 μg/mL) for 10 days and fixed with 4 % formaldehyde after 10 days. These minimally-prepared samples were dehydrated in ethanol, sputter coated with 20 nm of platinum for charge dissipation, and cross-sectioned using gallium ions in a dual beam instrument. Cross sections of minimally-prepared samples reveal nanoparticles internal to the progenitor cells. The nanoparticles are found within three dimensional clusters inside of the cells. After these clusters of nanoparticles were located during the cross-sectioning process, the locations were marked so that they can be located for correlated optical and helium ion microscopy. Helium ion microscopy confirmed the presence of individual gold nanoparticles of 30 nm diameter within the cells (see figure 1).

In contrast to minimally-prepared samples, cells on sapphire discs were exposed to 10 nm gold particles (5 μg/mL) for 3 days and preserved by high pressure freezing after 10 days. These samples were cryogenically protected, freeze substituted, embedded in epoxy, sectioned and stained for electron microscopy. The finished sections were imaged at tilt angles from -60 to 60 degrees in 1 degree intervals. The tilt-series were acquired using SerialEM [2]. A 3-D tomogram from the tilt-series was calculated with R-weighted back projection using IMOD [3]. The tomogram clearly shows a cluster of

gold particles, along with detail of the cellular structure (see figure 2).

In conclusion, through correlative techniques we clearly demonstrate that gold nanoparticles with diameters of 10 nm and 30 nm are internalized by rat cortex neural progenitor cells. In addition, we show with minimal sample preparation particles can be located within cells by focused ion beam cross-sectioning. These particles appear prominently in scanning electron and helium ion microscopies. The gold particles retain their shape over the course of the 10 day exposure and are typically found in clusters near the progenitor cell membrane.

References

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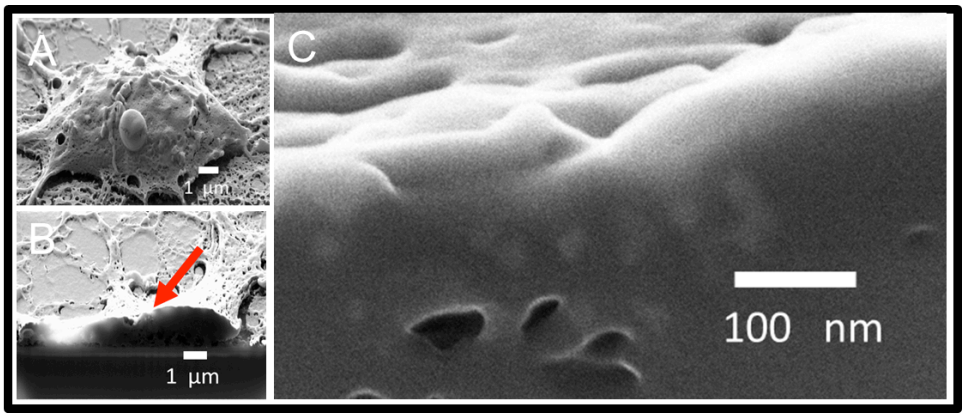

Figure 1. **A.** SEM image of a cell before focused ion beam (FIB) cross-sectioning. **B.** The red arrow points to particles found inside the cell after FIB cross-sectioning. **C.** Helium ion micrograph showing individual particles inside the cell.

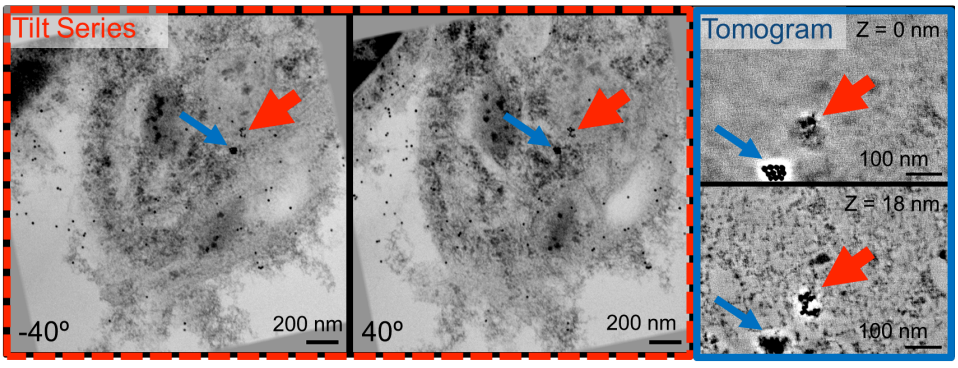

Figure 2. Tilt-series and resulting tomogram. The cell was stained with osmium tetroxide and potassium ferrous cyanide revealing its inner structure. A cluster of 10 nm nanoparticles (large red arrow) is visible in the bulk of the section. The tomogram is reconstructed with the aid of 15 nm gold fiducials placed on the surface of the section (small blue arrow).