In situ electron microscopy of bio-nano interfaces

Ilett, M.[1], Bamiduro, F.[2], Matar, O.[2], Brown, A.[2], Brydson, R.[2] and Hondow, N.[2]

[1] School of Chemical and Process Engineering, University of Leeds, United Kingdom, [2] University of Leeds, United Kingdom

Continued research into the applied use of nanoparticles within life sciences demands a fundamental understanding of the behaviour of nanoparticles within different biological media. Nanoparticle dispersions relevant to medical or toxicological studies are complex systems, with numerous biological and inorganic components, and while transmission electron microscopy (TEM) can be used as a nanoscale characterisation tool, several factors need to be considered. Firstly, the preparation of nanoparticle dispersions frequently employs abrasive techniques such as ultra-sonication, and often there is little consideration of the affect these methods can have on nanoparticle characteristics. Secondly, representative analytical TEM needs to be carried out on samples in the native state. *In situ* TEM is now in a rapid state of growth with cryogenic-analytical scanning TEM (cryo-analytical STEM) and liquid cell TEM at the forefront of research. We aim to compare these two techniques whilst establishing any effects of suspension preparation.

An investigation into four nanoparticles; $BaTiO_3$, ZnO, Fe_2O_3 and Au, that have already shown promise within nanomedicine has been carried out. The nanoparticles were dispersed in media typical of those used in common *in vitro* studies, Dulbecco's Modified Eagles Medium and Roswell Park Memorial Institute cell culture media, supplemented with fetal bovine serum. In analysis of these systems we employed novel cryo-analytical STEM using both energy dispersive X-ray (EDX) spectroscopy and electron energy loss spectroscopy (EELS) to image and analyse suspensions in the native state. EDX was used to identify and spatially map different elements and EELS was used for elemental and oxidation state analysis.

Significant findings showed that upon dispersion of nanoparticles within cell culture media via bath sonication, a calcium and phosphorus rich coating formed around all four nanoparticles tested (Figure 1). The formation of this coating was independent of surface charge, size or shape and believed to be entirely an artefact of the effects from bath sonication, specifically the pH change induced by the elevated temperatures. We can also show that this is only identifiable through cryo-analytical STEM, since conventional drop cast TEM sample preparation led to artefacts from sample drying (1). To enable representative analysis of frozen specimens, we successfully established suitable acquisition conditions for cryo-analytical EDX and EELS analysis and now intend to compare this technique to liquid cell TEM to ascertain whether there are any important differences or similarities between the two *in situ* systems.

To conclude, significant interactions occurring at the bio-nano interface are seen to arise with the belief that this will have demonstrable effects on nanoparticle behaviour in biological studies. These interactions are in some cases driven solely by the sample preparation technique used, highlighting the pressing need for thorough preliminary work to ensure any sample preparation artefacts are acknowledged. It is vital that these artefacts are understood to ensure conclusions regarding how nanoparticle behaviour within biological media relates to the inherent characteristics of the particle are not misled.

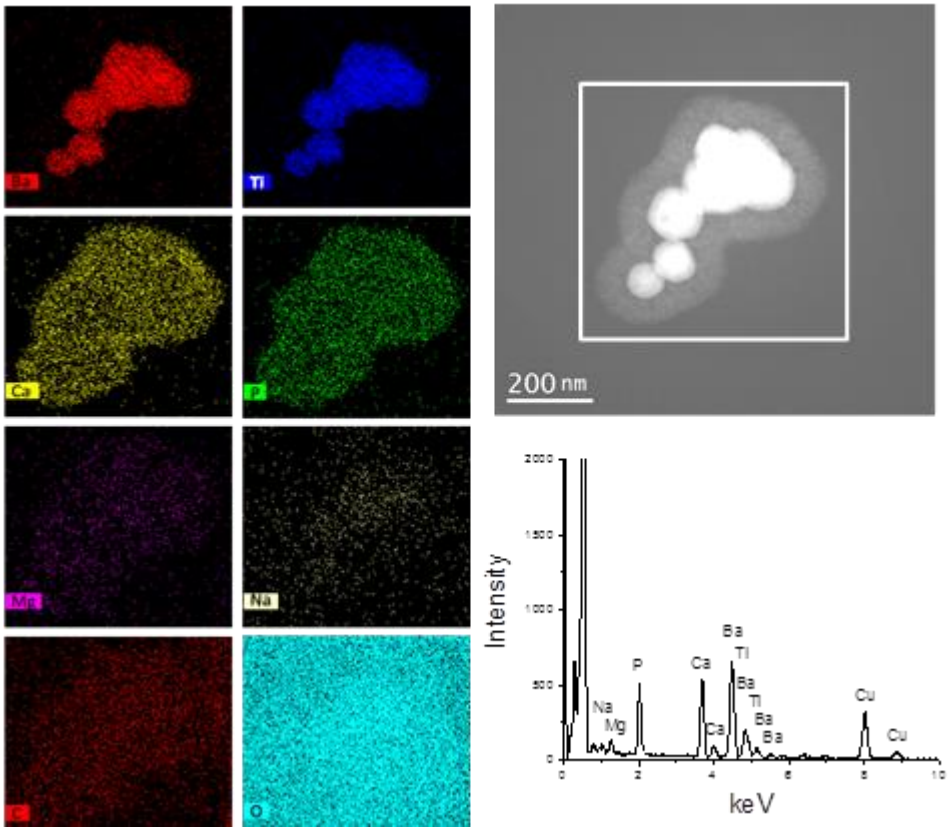

Figure 1: Cryo-analytical STEM of $BaTiO_3$ nanoparticles dispersed in Roswell Park Memorial Institute + 10% Fetal Bovine Serum. The HAADF STEM image (top right) shows a clear coating has established around the nanoparticles. The EDX spectrum (bottom right) indicated strong signals for Ca and P and EDX mapping (left) showed these elements spatially resolved to the position of the coating seen in the HAADF STEM image. Other constituents of the media (Na and Mg) were fully dispersed and Ba and Ti spatially resolved to the position of the nanoparticles in the image as expected.

References

1. Ilett, M. et al. Cryo-STEM-EDX spectroscopy for the characterisation of nanoparticles in cell culture media. *Journal of Physics: Conference Series.* 2017, **902**(1), p.012006.