Serum albumin enhances the membrane activity of ZnO nanoparticles†

Adam H. Churchman,* Rachel Wallace,* Steven J. Milne,* Andy P. Brown,* Rik Brydson* and Paul A. Beales***

We investigate the effect of serum albumin on the interaction of ZnO nanoparticles with DOPC lipid membranes and show that the size-stabilizing effect of the protein corona enhances their interaction with lipid membranes, which manifests, in part, as an increased ordering in the lipid packing.

Formation of protein coronas around engineered nanoparticles (NPs) has been shown to have a significant impact on how these materials interact with biology. This has profound implications for the potential toxicity of nanomaterials and their biomedical applications such as therapeutic delivery and diagnostic imaging.

For example, protein coronas formed in serum have been shown to reduce cell uptake and toxicity of several classes of NP. However, serum has been found not to reduce the toxicity of ZnO NPs to human breast cancer cell line. It is becoming clear that understanding the effect of serum proteins within toxicity assays is an important challenge in resolving apparently contradictory reports of nanomaterial toxicity.

To gain a deeper fundamental understanding of the physical nature of these nano–bio interactions, minimal model systems with a small, known number of constituents need to be employed. Such minimal systems offer unique opportunities to isolate and investigate passive interaction mechanisms. Of particular interest is the ability of NPs to cross biological barriers, which in part determines their eventual biodistribution. For example, the interaction of NPs with plasma membranes regulates their access to the interior biomolecular machinery of cells. The structural matrix of a cell membrane consists of a lipid bilayer, which can be reconstituted into model biomembranes in the form of unsupported vesicles for the purpose of fundamental biophysical analysis. Interactions of NPs with lipid membranes reveal rich phenomenology, even within such minimal systems, where interactions are highly dependent upon NP properties such as polarity, charge, shape and size.

Here, we investigate the interaction of ZnO NPs with model biomembranes composed of the common phospholipid dioleoyl phosphocholine (DOPC). ZnO NPs are commonly used in coatings, pigments and sunscreens and despite its solubility is generally shown not to be toxic unless inhaled. When however ZnO NPs do penetrate cells it has recently been reported that they may be carcinogenic as a result of causing DNA damage. Therefore work towards understanding the interactions of ZnO NPs at the cell membrane, which may allow them to gain access to the cell interior, will be important in developing particle engineering strategies that passivate these materials against cell uptake (or to optimise uptake for therapeutic purposes, e.g. cancer therapy).

To mimic the effects of a primitive protein corona, our studies are conducted with or without the presence of serum albumin (bovine; BSA), by far the most abundant protein in blood serum. ZnO NP samples prepared with BSA were created from an initial stock solution of 10 mg ZnO NPs dispersed within 1 ml of 100 mg ml⁻¹ BSA in water. This concentration of BSA is comparable to the total protein concentration in blood serum, approximately half of which is serum albumin. This stock solution was diluted into experimental samples as required.

We find that the presence of BSA significantly increases morphological transitions of lipid membranes resulting from addition of ZnO NPs. The primary membrane models we utilise are giant unilamellar vesicles (GUVs), which allow direct optical observation of membrane perturbation by NPs due to their microscale architecture. DOPC GUVs were formed by electroformation and contained a trace component (0.5 mol%) of Rhodamine-labelled lipid (Rh-DOPE). Confocal fluorescence microscopy was used to image morphology changes in GUVs induced by ZnO NPs. Control experiments confirmed no significant impact on GUV morphology is caused by BSA alone or Zn²⁺ ions (5.6 mM ZnSO₄; equivalent to the maximum total Zn concentration in our ZnO NP experiments). Fig. 1a shows GUVs in the presence of 420 μg ml⁻¹ ZnO NPs (no BSA); little change in morphology was observed except for the occasional lipid tubule protruding from/into some GUVs. Conversely when the same concentration of ZnO NPs in the presence of 4.2 mg ml⁻¹ BSA is added to GUVs, several significant morphological responses were seen (Fig. 1b–d).

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‡ Centre for Molecular Nanoscience, School of Chemistry, University of Leeds, LS2 9JT, UK. E-mail: p.a.beales@leeds.ac.uk

‡ Institute for Materials Research, SPEME, University of Leeds, LS2 9JT, UK

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The overall interaction appears to be highly complex with aggregation of GUVs (Fig. 1b), large numbers of lipid nanotubes ejected from the membrane (Fig. 1c) and fission processes into smaller GUVs (Fig. 1d). Despite these dramatic observations, the permeability barrier of the membrane surprisingly remains largely intact. 10 kDa Alexa Fluor 647 dextran (blue; A647-10k) acting as a passive leakage marker in the external environment of the GUVs remains excluded from the interior lumen of > 95% of GUVs (Fig. 1). The conservation of the membranes’ barrier properties strongly implies that these NPs do not passively translocate into the interior lumen of the GUVs and thus only directly interact with the outer lipid monolayer of the GUVs. The resultant asymmetry of NPs across the membrane may therefore lead to curvature effects that drive the observed morphological changes, similar to previous reports.16

In the absence of BSA, large darker regions were seen to increase in the background of GUV images, which we interpret to be a result of ZnO NP aggregation. This would likely deplete NPs in the suspension, preventing significant interaction with GUV membranes. We investigate the stability of ZnO NP aqueous dispersions with and without BSA by dynamic light scattering (DLS) and transmission electron microscopy (TEM) (Fig. 2). TEM samples were prepared by thin film plunge freezing before sublimation of vitrified water under vacuum, preventing significant interaction with GUV membranes. We investigated the stability of ZnO NP dispersions, the 440 nm peak increases significantly with respect to 490 nm spectral peak, indicating increased ordering of lipids in the membrane. However, without BSA, the intensities at 440 nm and 490 nm both reduce fairly concurrently implying no significant change in lipid ordering but the decrease in overall intensity perhaps relates to adsorption of lipid onto ZnO NP aggregates, removing some of the LUVs from the sample.

The detected increase in lipid ordering in the presence of BSA-stabilized ZnO NPs is confirmed by multiphoton fluorescence imaging of GUVs containing Laurdan. The normalised ratiometric intensity of the two emission maxima of Laurdan is known as the generalised polarisation function, GP = (I_{440} - I_{490})/(I_{440} + I_{490}) where I, is the fluorescence intensity at x nm. Higher GP values imply a more ordered, less hydrated lipid membrane. Multiphoton...
work is required to determine if there is a direct link between the ordering of lipids by NPs and the well established efficacy of NPs to cross plasma membranes and access the cell’s interior workings. It will also be necessary to improve our understanding of the coupling between particle type, protein corona structure (e.g. exposed functional groups) and the interaction with model biomembranes. AHC acknowledges the support of Nuffield Foundation Research Bursary URB/39543. PAB thanks the Biomedical and Health Research Centre at the University of Leeds for funding and support. This work is also supported by the ENNSATOX programme funded by EU FP7 under grant agreement no. NMP-229244. The authors thank Dr Claus Svendson from the EU NANOFASTE programme for kindly supplying the ZnO nanoparticles. RW and AHC are grateful to Dr Nicole Hondow for help with the TEM plunge freezing. AHC and PAB thank Prof. Christoph Waitl for access to and assistance with the multiphoton microscope.

Notes and references