

ESI 2: Dissolution of NPs and MPs in cell culture medium using ICP-OES

The NP and MP dispersions in cell culture medium (pH 7.1) were prepared according to the same procedure as used for the preparation of the dispersions for the omics study. Dissolution of the NPs and MPs in cell culture medium was measured at various time intervals (0, 6, and 24 hrs). At each time interval, 10 mL of the dispersion was transferred to 3 kDa Millipore centrifuge tubes (Satorius, UK) and subjected to centrifugal ultrafiltration at 5000 g for 50 minutes to separate the dissolved fraction. Nitric acid (ICP grade) was added to the filtrate samples to a final concentration of 2% and samples were subsequently analysed using a Perkin Elmer ICP-OES Optima 8000 platform in order to assess how much, if any, dissolution of the NMs or MPs had occurred. Each sample was prepared and analysed in triplicate. Prior to acquisition the torch was aligned (radially and axially) using the Mn wavelengths and calibration curves were constructed using standard solutions of each element prepared by serial dilutions of 1000 ppm in 2% nitric acid. A calibration curve with R^2 value of >0.999 was deemed sufficient for analysis.

The concentrations of ions detected at each time point are presented in Table S2 below. Interestingly, the dissolved concentration from ZnO NPs determined at 24 hours in complete cell culture medium were higher than the dissolved concentrations determined at pH 7 in DI water (Papadiamantis *et al.*, manuscript in preparation), consistent with the known affinity of Zn^{2+} for biological components (protein, lysosomes etc.)¹ and its known tendency to form complexes with organic components in the long term²⁻⁴. Clearly, this would also be the case for the Zn^{2+} ions released from the ionic control, which would be similarly bound to biomolecules.

Table S2: Ion concentrations in cell culture medium after 0, 6 and 24 hours

Incubation (hours)→ Test material ↓	Initial MP or NM concentration (mg/L)	Ion concentration (µg/mL)		
	0	0	6	24
Ag NP-NM300K	38.6	0.075 +/- 0.01	0.093 +/- 0.03	0.040 +/- 0.003
Ag MP	128	0.005 +/- 0.0002	0.004 +/- 0.0001	0.005 +/- 0.0004
ZnO NP-NM110	15	1.401 +/- 0.06	1.378 +/- 0.07	1.411 +/- 0.01
ZnO NP- NM 111	10	0.439 +/- 0.02	0.899 +/-0.07	0.989 +/- 0.03
ZnO MP	30	1.323 +/- 0.22	1.338 +/- 0.10	1.461 +/- 0.03
CeO ₂ NP-A	128	<LOD	<LOD	<LOD
CeO ₂ NP-C	128	<LOD	<LOD	<LOD
CeO ₂ NP-E	128	<LOD	<LOD	<LOD
CeO ₂ NP-NM212	128	<LOD	<LOD	<LOD

References:

1. V. Frazzini, E. Rockabrand, E. Mocchegiani and S. L. Sensi. Oxidative stress and brain aging: is zinc the link? *Biogerontology*, 2006, **7**, 307-314.
2. M. Li, L. Zhu and D. Lin. Toxicity of ZnO nanoparticles to *Escherichia coli*: mechanism and the influence of medium components. *Environ Sci Technol*, 2011, **45**, 1977-1983.
3. R. B. Reed, D. A. Ladner, C. P. Higgins, P. Westerhoff and J. F. Ranville. Solubility of nano-zinc oxide in environmentally and biologically important matrices. *Environ Toxicol Chem*, 2012, **31**, 93-99.
4. T. Xia, M. Kovochich, M. Liong, L. Madler, B. Gilbert, H. Shi, J. I. Yeh, J. I. Zink and A. E. Nel. Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. *ACS Nano*, 2008, **2**, 2121-2134.

Supplementary material of:

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