Supplementary Information

Challenge to assess the toxic contribution of metal cation released from nanomaterials for nanotoxicology – a case of ZnO nanoparticles

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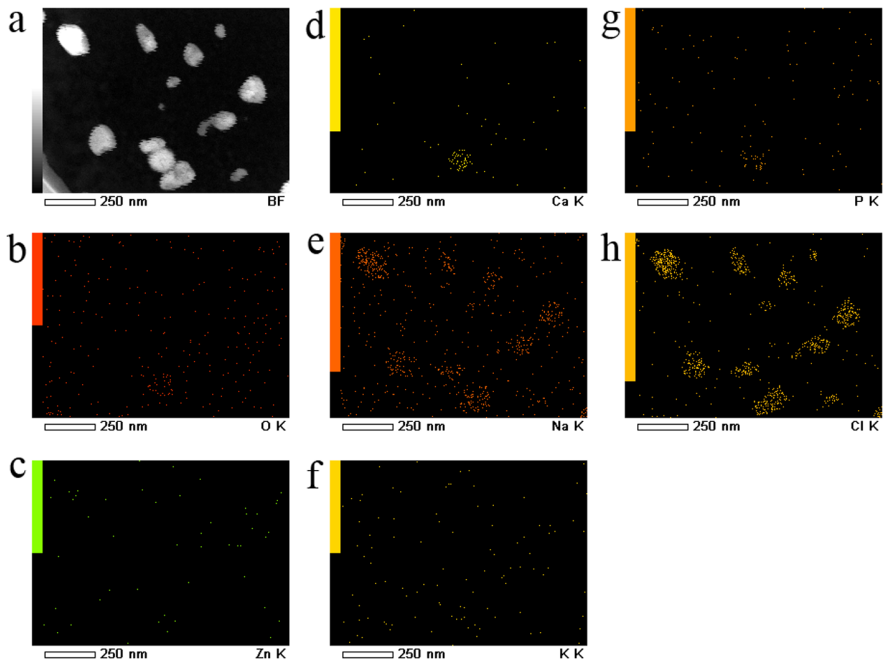

Figure S1. Formation of aggregates/agglomerates of DMEM medium with addition of fetal bovine serum but without ZnO NPs. **a**, TEM image showing where the elemental maps were obtained. **b**, TEM/EDS O-K map. **c**, TEM/EDS Zn-K map. **d**, TEM/EDS Ca-K map. **e**, TEM/EDS Na-K map. **f**, TEM/EDS K-K map. **g**, TEM/EDS P-K map. **h**, TEM/EDS Cl-K map. No Zn elemental signal was detected.

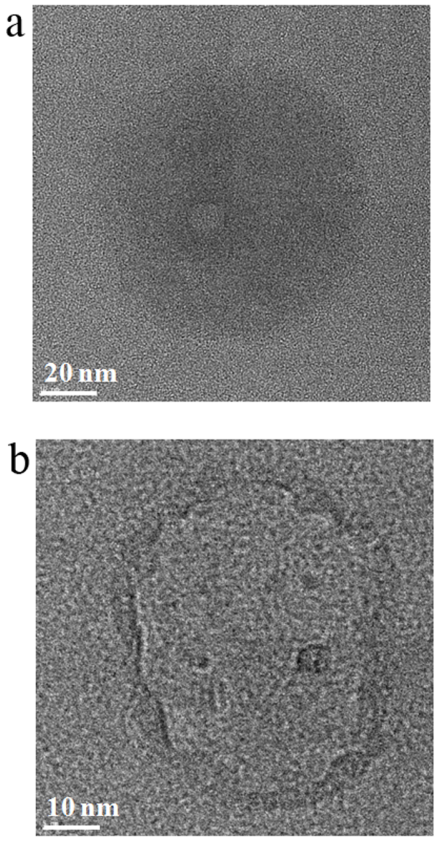

Figure S2. High-resolution TEM images. **a**, Obtained from aggregates/agglomerates of DMEM medium with addition of fetal bovine serum but without ZnO NPs. **b**, Obtained from aggregates/agglomerates of PBS but without ZnO NPs. No crystalline structure is observed.

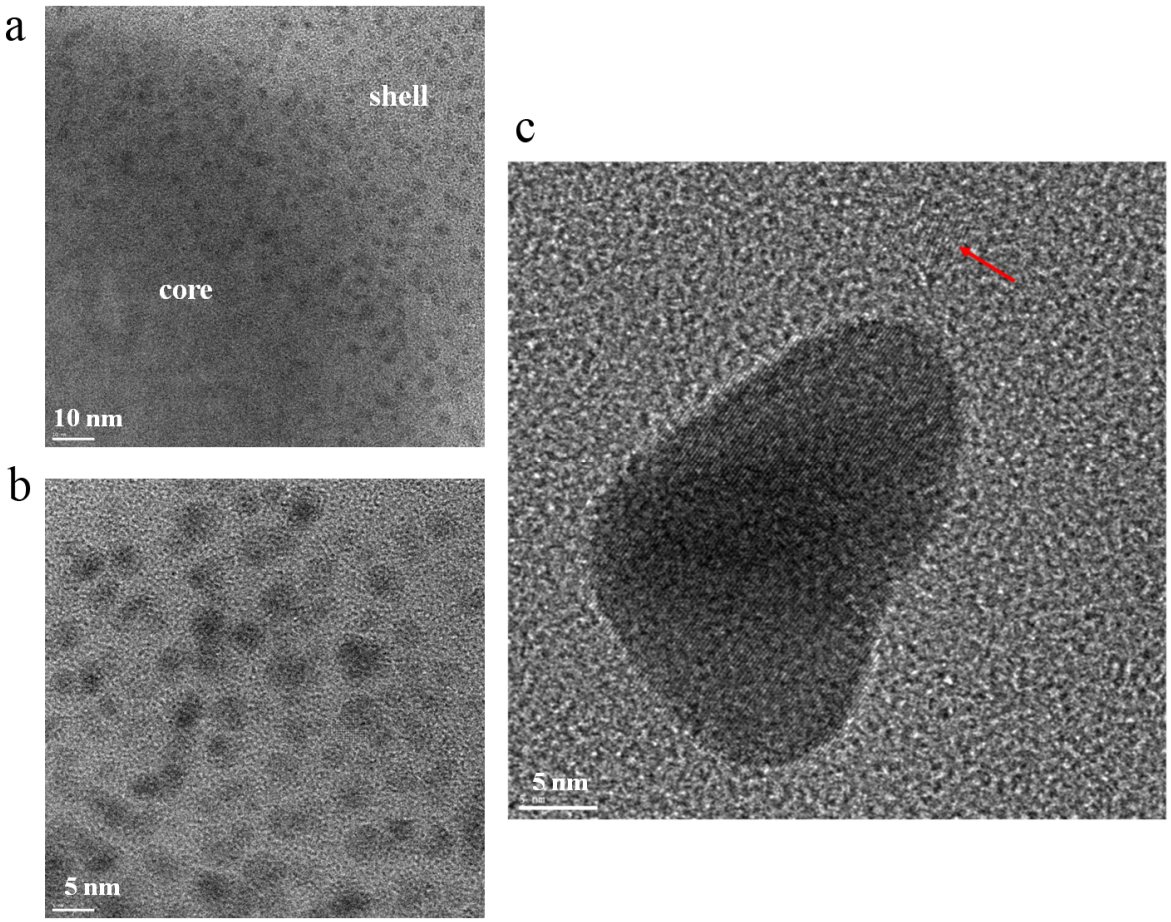

Figure S3. High-resolution TEM images obtained from the supernatant collected after centrifugation of the ZnO suspension in medium with fetal bovine serum, showing small ZnO NPs embedded in the shell. Image (b) was obtained from the shell region of image (a).

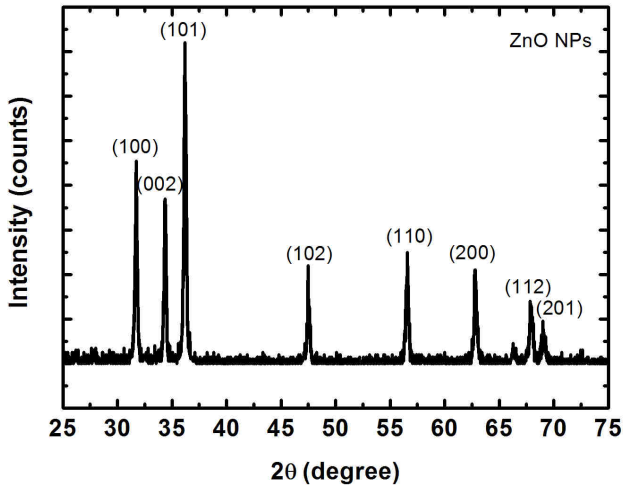

Figure S4. X-ray powder diffraction (XRD) patterns for ZnO NPs. XRD was carried out on a Rigaku X-Ray Diffractometer Ultima III at a scanning rate of 3°/min in the 2θ range from 10° to 90° with sampling of 0.02°. The ZnO NPs were dispersed in the DMEM medium with FBS for XRD measurement. We also measured the ZnO NPs powders by XRD. The XRD patterns are the same as that of ZnO NPs dispersed in the medium with FBS. We did not detect diffraction peaks for the medium with or without FBS.

Table S1. Hydrodynamic size distribution of ZnO NP (60 nm) suspensions

	Solvent	medium + FBS	medium	Supernatant		
				b	c	d
Size[e] (nm)	Peak 1	11 657.7 (3047.5) [28.6%]	24 111.6 (3567.6) [31.8%]	1611.5 (137.6) [47.3%]	176.5 (23.6) [14.0%]	160.2 (13.7) [17.1%]
	Peak 2	1158.4 (415.9) [42.0%]	1531.8 (302.2) [68.2%]	19.8 (1.7) [52.7%]	23.8 (11.1) [59.0%]	17.8 (1.9) [48.8%]
	Peak 3	196.6 (31.4) [6.0%]			7.4 (1.5) [11.9%]	4.2 (0.7) [34.1%]
	Peak 4	10.9 (1.3) [23.4%]			2.2 (0.2) [15.1%]	
	Total	3893.2 (5282.4)	8722.2 (10 713.)	774.9 (800.4)	40.7 (58.8)	39.7 (56.6)

Just before measurement, samples were stirred by Ecan TubeMixer for 1 min. The initial concentration of ZnO suspensions was about 25 µg/mL.

[b] The supernatant was collected after centrifugation of ZnO suspension in medium with FBS at 20,000 × *g* for 20 min.

[c] The supernatant was collected after centrifugation of ZnO suspension in medium with FBS at 150,000 × *g* for 20 min.

[d] The supernatant was collected after centrifugation of ZnO suspension in medium with FBS at 150,000 × *g* for 60 min.

[e] Size expressed as average (standard deviation) [fraction]

No signal was obtained from the medium with FBS solution. The average size of ZnO NPs is about 60 nm, but the size is not uniform.

Table S2. Hydrodynamic size distribution of ZnO NP (~20 nm) suspensions

	Solvent	medium + FBS	medium	Supernatant
	Peak 1	1845.3 (156.5) [88.8%]	19513.1 (1669.6) [29.3%]	48.4 (17.5) [41.1%]
Size[a]	Peak 2	225.4 (74.8) [8.0%]	1592.7 (325.5) [70.7%]	9.4 (2.7) [58.9%]
(nm)	Peak 3	23.0 (2.4) [3.2%]		
	Peak 4			
	Total	349.2 (451.6)	6674.8 (8130.9)	25.8 (22.4)

Just before measurement, samples were stirred by Ecan TubeMixer for 1 min. The initial concentration of ZnO suspensions was about 25 µg/mL.

The supernatant was collected after centrifugation of ZnO suspension in medium with FBS at 150,000 × *g* for 60 min.

[a] Size expressed as average (standard deviation) [fraction]

No signal was obtained from the medium with FBS solution. The average size of ZnO NPs is about 20 nm, but the size is not uniform.