Supplementary Information

Formation of Nano-Bio-Complex as Nanomaterials Dispersed in a Biological Solution for Understanding Nanobiological Interactions

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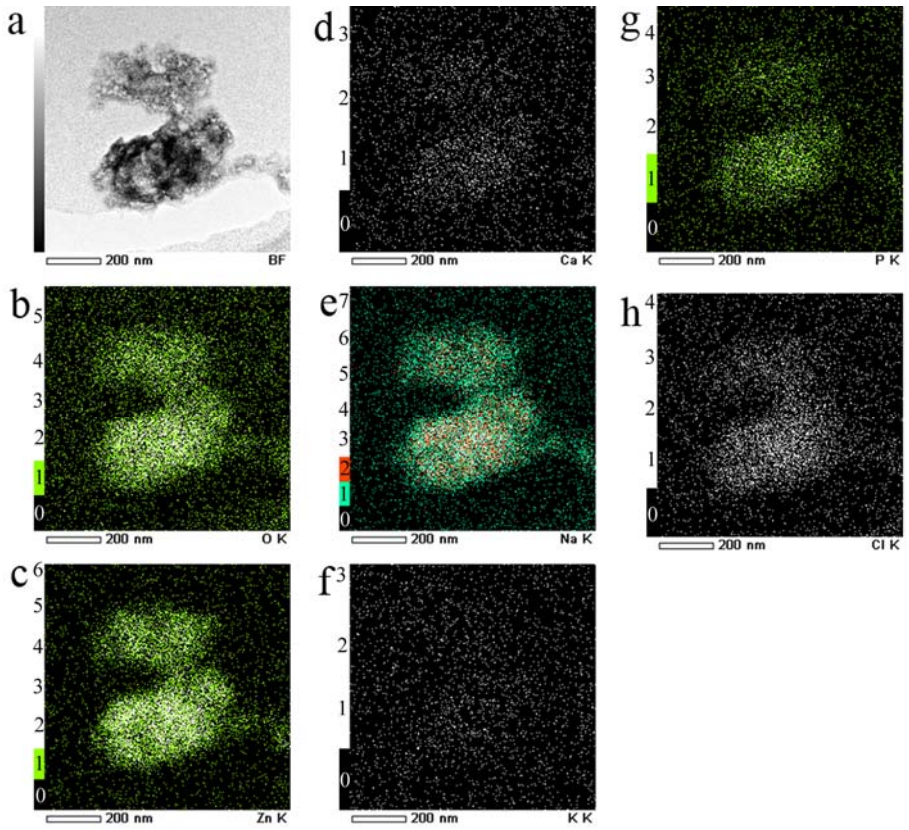

Figure S1. Formation of ZnO nanoparticle bio-conjugates dispersed in DMEM medium with addition of fetal bovine serum (~25 μg/mL). (a) TEM image showing where the elemental maps were obtained; (b) TEM/EDS O-K map; (c) TEM/EDS Zn-K map; (d) TEM/EDS Ca-K map; (e) TEM/EDS Na-K map; (f) TEM/EDS K-K map; (g) TEM/EDS P-K map; (h) TEM/EDS Cl-K map.

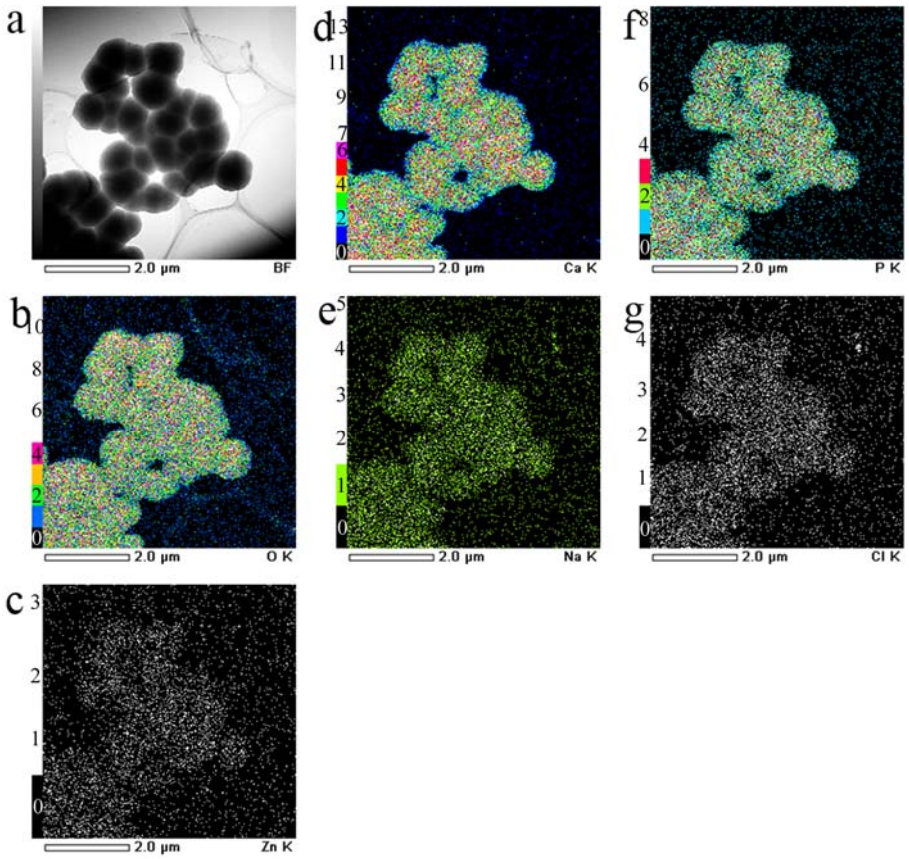

Figure S2. Formation of ZnO nanoparticle bio-conjugates dispersed in PBS with addition of fetal bovine serum (~25 µg/mL). (a) TEM image showing where the elemental maps were obtained; (b) TEM/EDS O-K map; (c) TEM/EDS Zn-K map; (d) TEM/EDS Ca-K map; (e) TEM/EDS Na-K map; (f) TEM/EDS P-K map; (g) TEM/EDS Cl-K map.

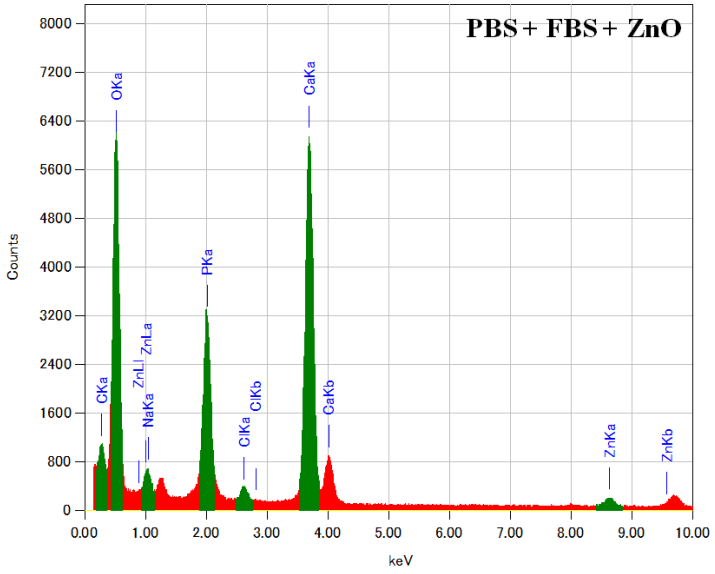

Figure S3. Energy dispersive spectroscopy (EDS) collected from the region in Figure S2.

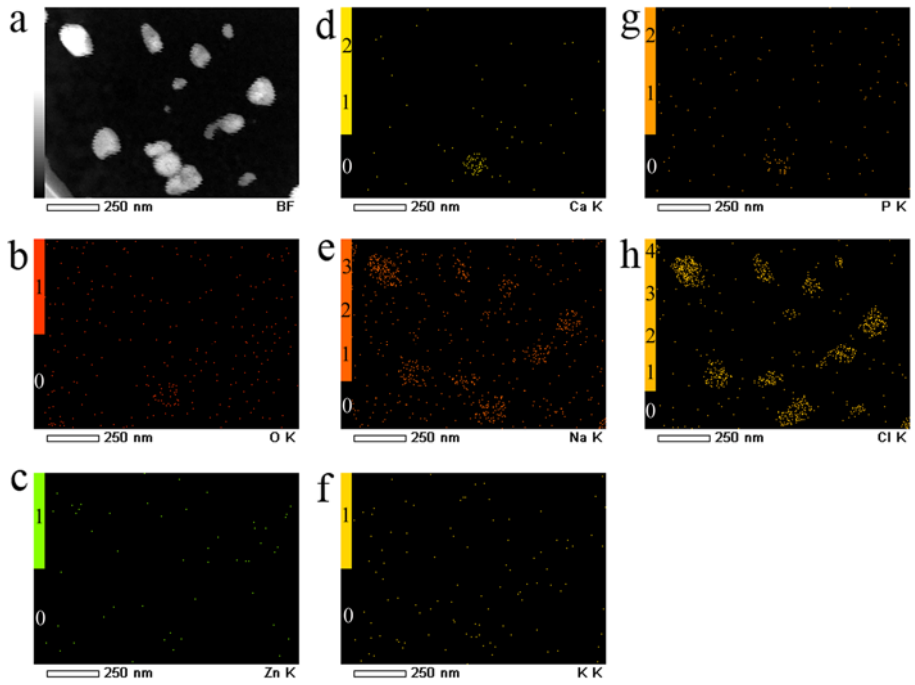

Figure S4. Formation of aggregates/agglomerates of DMEM medium with addition of fetal bovine serum but without ZnO NPs. (a) TEM image showing where the elemental maps were obtained; (b) TEM/EDS O-K map; (c) TEM/EDS Zn-K map; (d) TEM/EDS Ca-K map; (e) TEM/EDS Na-K map; (f) TEM/EDS K-K map; (g) TEM/EDS P-K map; (h) TEM/EDS Cl-K map. No Zn elemental signal was detected.

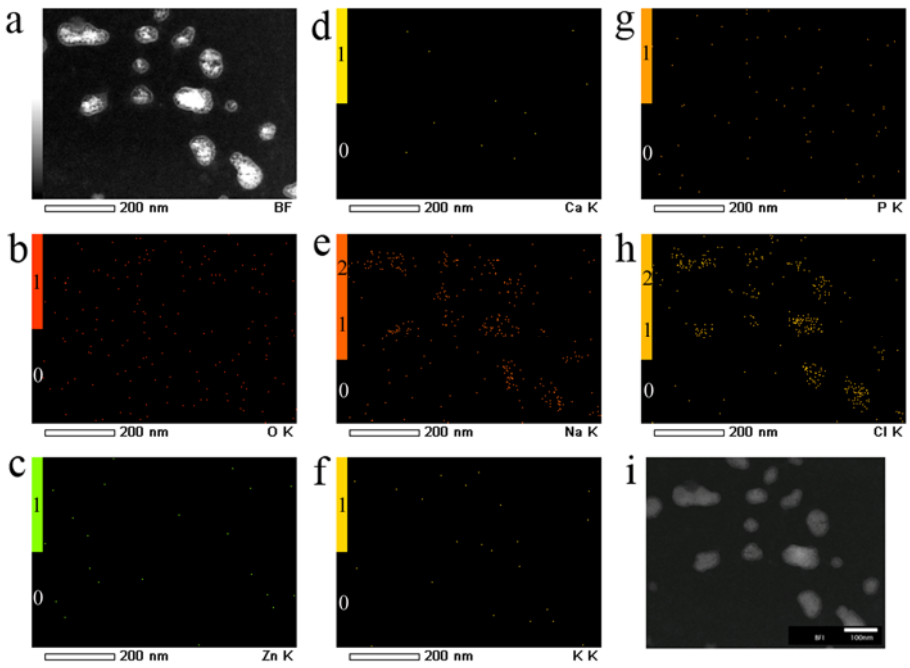

Figure S5. Formation of aggregates/agglomerates of PBS solution (comprised of $NaHPO_4$, Na_2HPO_4, and NaCl) but without ZnO NPs. (a) TEM image showing where the elemental maps were obtained; (b) TEM/EDS O-K map; (c) TEM/EDS Zn-K map; (d) TEM/EDS Ca-K map; (e) TEM/EDS Na-K map; (f) TEM/EDS K-K map; (g) TEM/EDS P-K map; (h) TEM/EDS Cl-K map; (i) TEM image obtained before elemental mapping. Comparison between (a) and (i), we found that the clusters are easily damaged by electron beam of the TEM measurement.

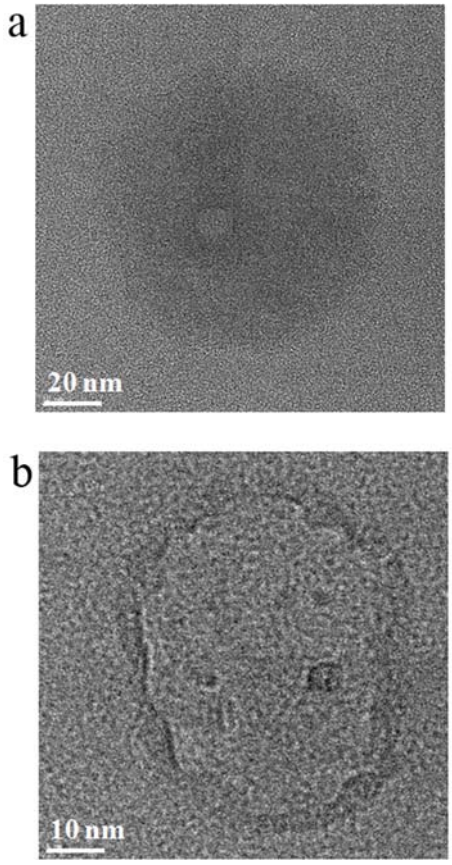

Figure S6. High-resolution TEM images. (a) Obtained from aggregates/agglomerates of DMEM medium with addition of fetal bovine serum but without ZnO NPs; (b) Obtained from aggregates/agglomerates of PBS but without ZnO NPs. No crystalline structure is observed.

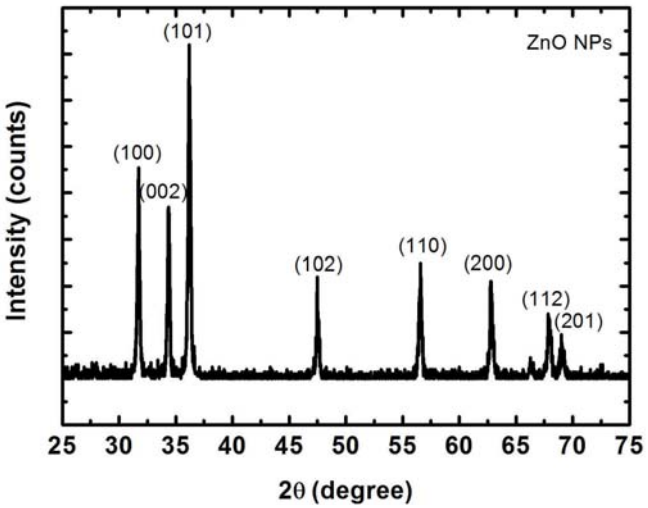

Figure S7. X-ray powder diffraction (XRD) patterns for ZnO nanoparticles. XRD was carried out on a Rigaku X-Ray Diffractometer Ultima III at a scanning rate of 3°/min in the 2θ range from 10° to 90° with sampling of 0.02°.

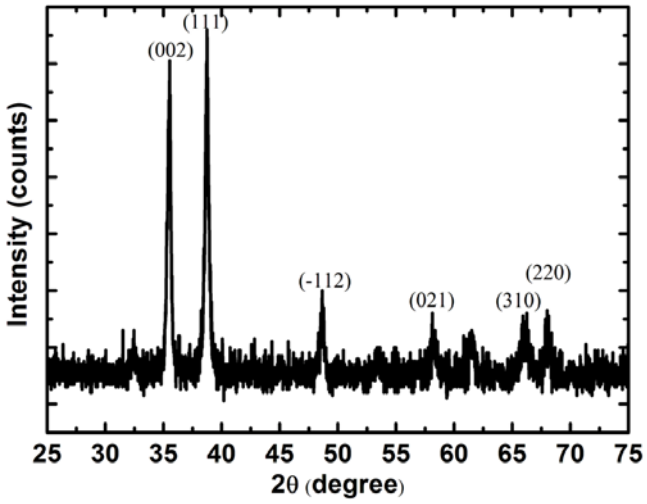

Figure S8. X-ray powder diffraction (XRD) patterns for CuO nanoparticles. XRD was carried out on a Rigaku X-Ray Diffractometer Ultima III at a scanning rate of 3°/min in the 2θ range from 10° to 90° with sampling of 0.02°.

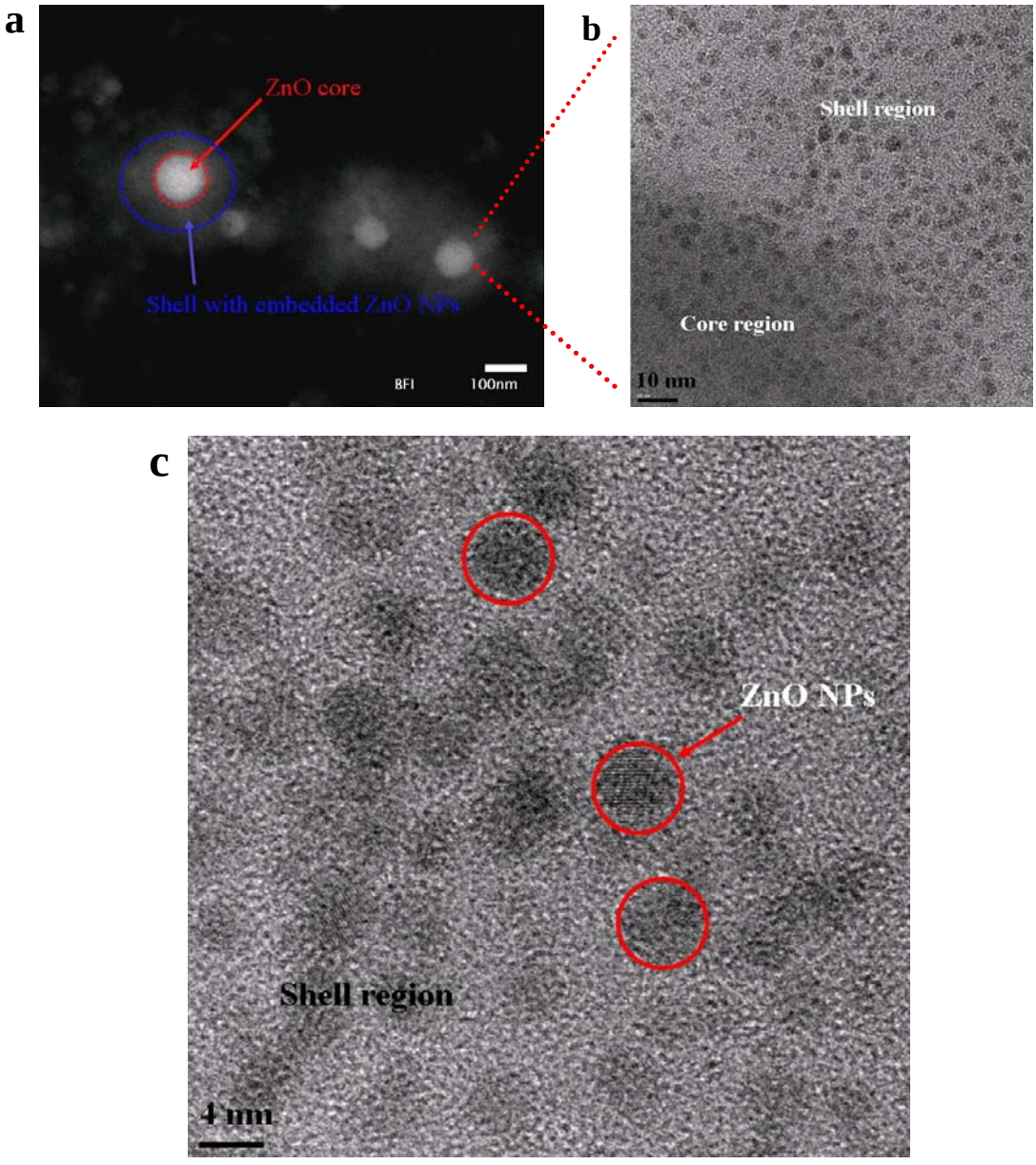

Figure S9 TEM images of ZnO NP bio-complexes. (a) Dark field TEM image showing a large sample area; (b) and (c) High-resolution TEM images showing crystalline ZnO NPs (~ 5 nm) embedded in the shell region of the bio-complexes. The density of ZnO NPs in the core region is much higher so as to be regarded as core region. In this investigation, the averaged primary size of the ZnO NP is ~20 nm, and we further removed large ZnO clusters by centrifugation.

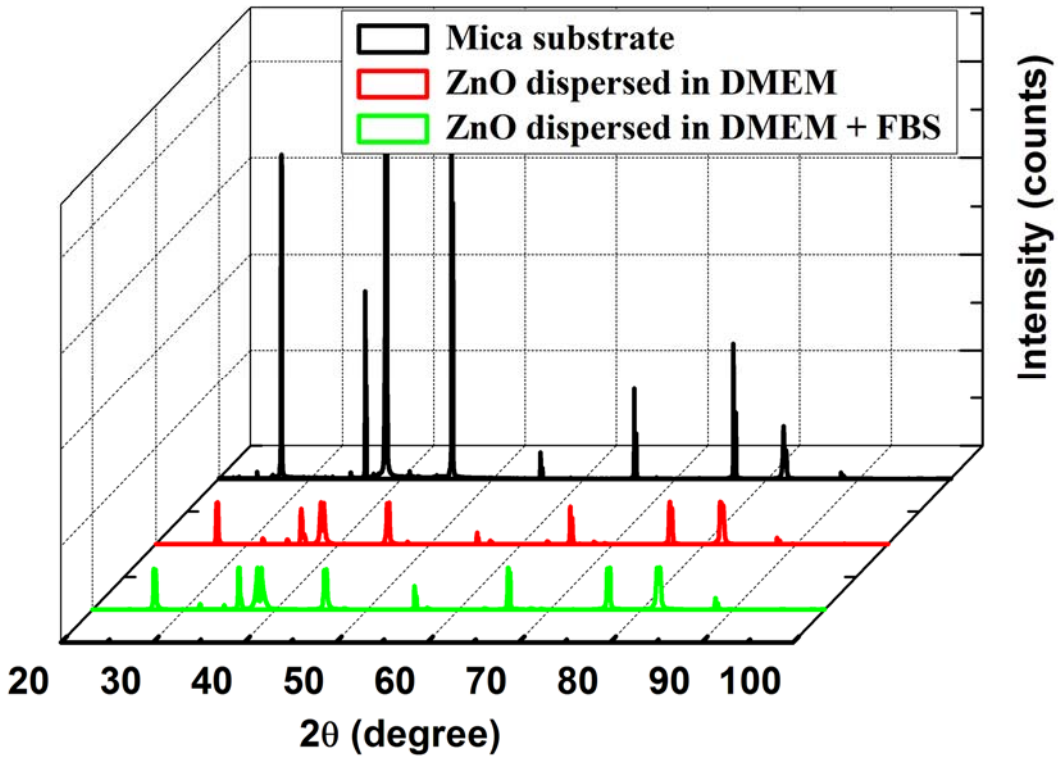

Figure S10. XRD patterns for ZnO samples. The preparation of the samples for XRD measurement was as follows. First, the ZnO nanoparticles were dispersed into DMEM or DMEM + FBS. Second, a mica/Au substrate was immersed into the ZnO nanoparticles suspension. Third, the mica/Au substrate was taken out of the suspension and tried for XRD measurement. We observed ZnO patterns and the patterns originated from the mica substrate on the ZnO nanoparticles dispersed in DMEM or DMEM plus FBS. No signal associated with the DMEM and FBS was detected. In the case of the ZnO nanoparticles dispersed in the DMEM plus FBS, we also detected Au(111) pattern located at 2θ of 38.187°. The XRD measurement was carried out on a Rigaku X-Ray Diffractometer (Rigaku Rint 2500) at a scanning rate of 1°/min in the 2θ range from 10° to 100° with sampling of 0.02°.

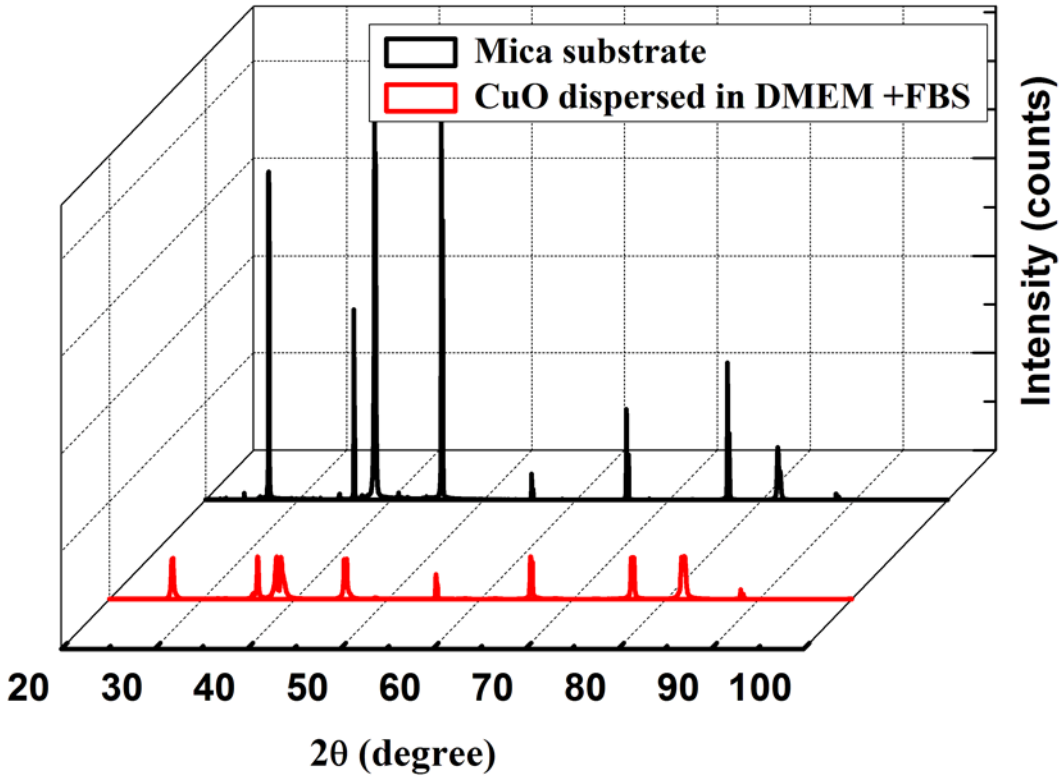

Figure S11. XRD patterns for CuO samples. The preparation of the samples for XRD measurement was as follows. First, the CuO nanoparticles were dispersed into DMEM + FBS. Second, a mica/Au substrate was immersed into the CuO nanoparticles suspension. Third, the mica/Au substrate was taken out of the suspension and tried for XRD measurement. We observed CuO patterns and the patterns originated from the mica substrate on the CuO nanoparticles dispersed in DMEM plus FBS. No signal associated with the DMEM and FBS was detected. We also detected Au(111) pattern located at 2θ of 38.187°. The XRD measurement was carried out on a Rigaku X-Ray Diffractometer (Rigaku Rint 2500) at a scanning rate of 1°/min in the 2θ range from 10° to 100° with sampling of 0.02°.

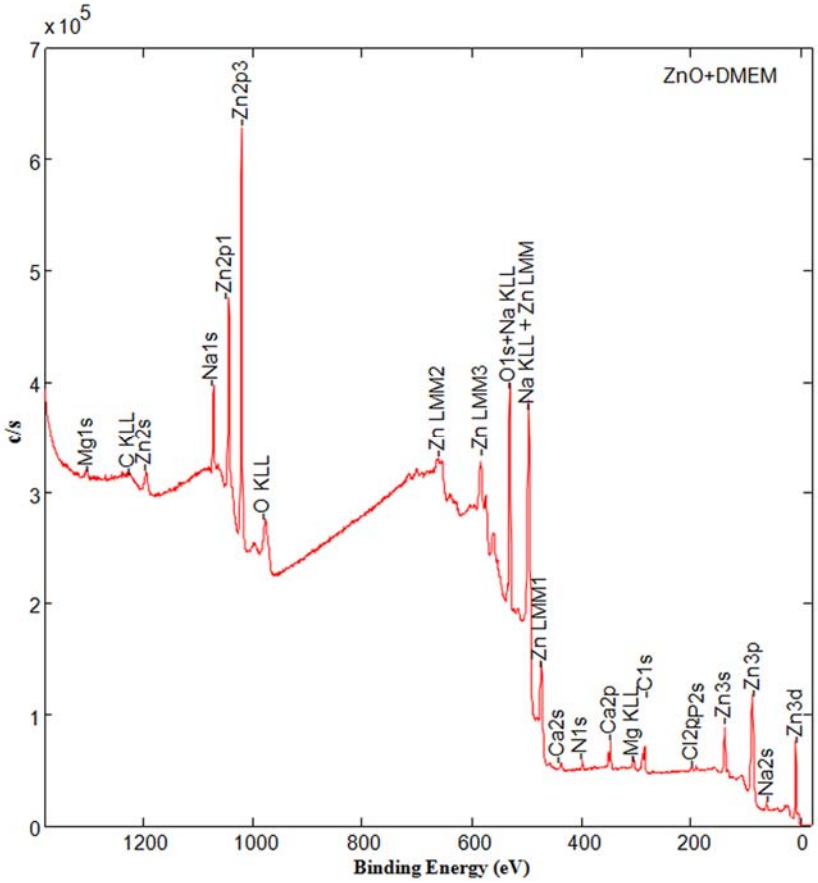

Figure S12. Survey XPS spectrum of ZnO NPs dispersed in DMEM.

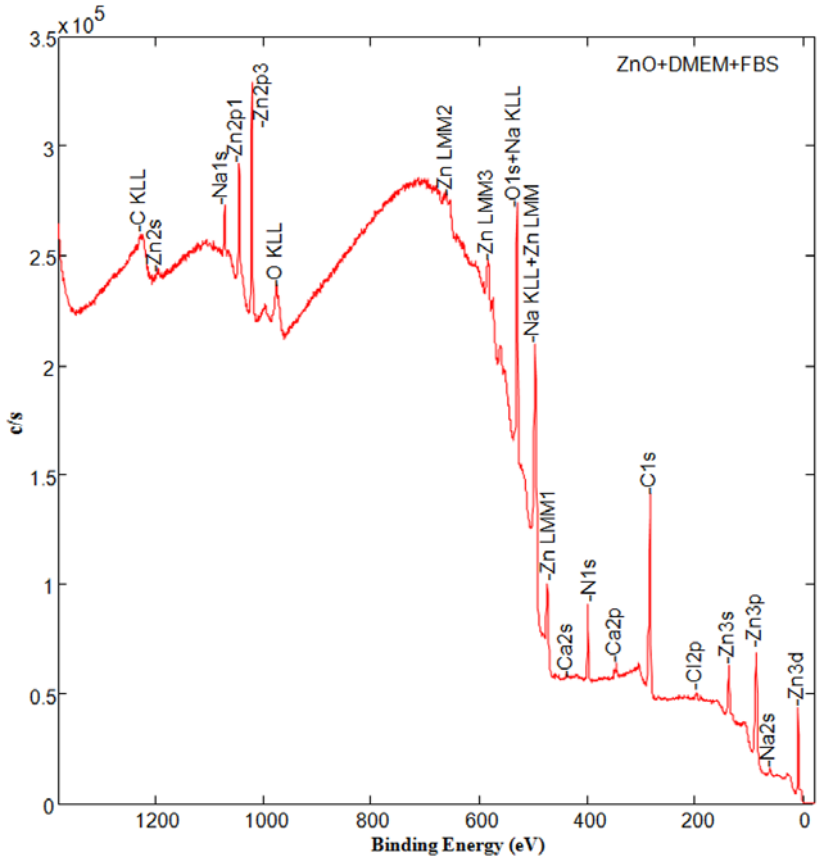

Figure S13. Survey XPS spectrum of ZnO NPs dispersed in DMEM with FBS.

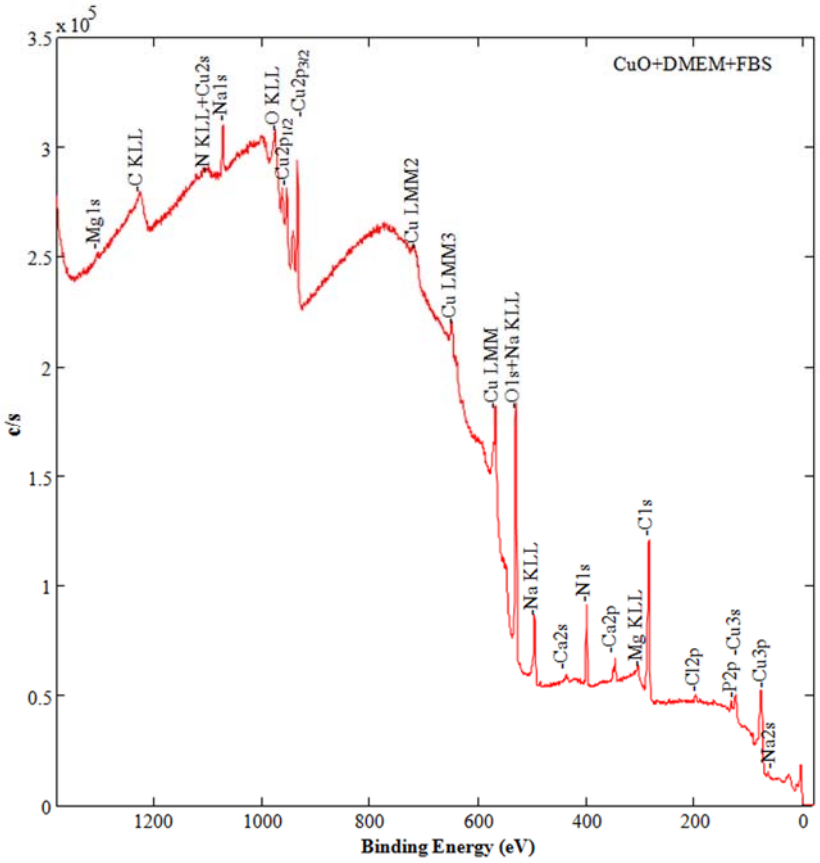

Figure S14.Survey XPS spectrum of CuO NPs dispersed in DMEM with FBS.

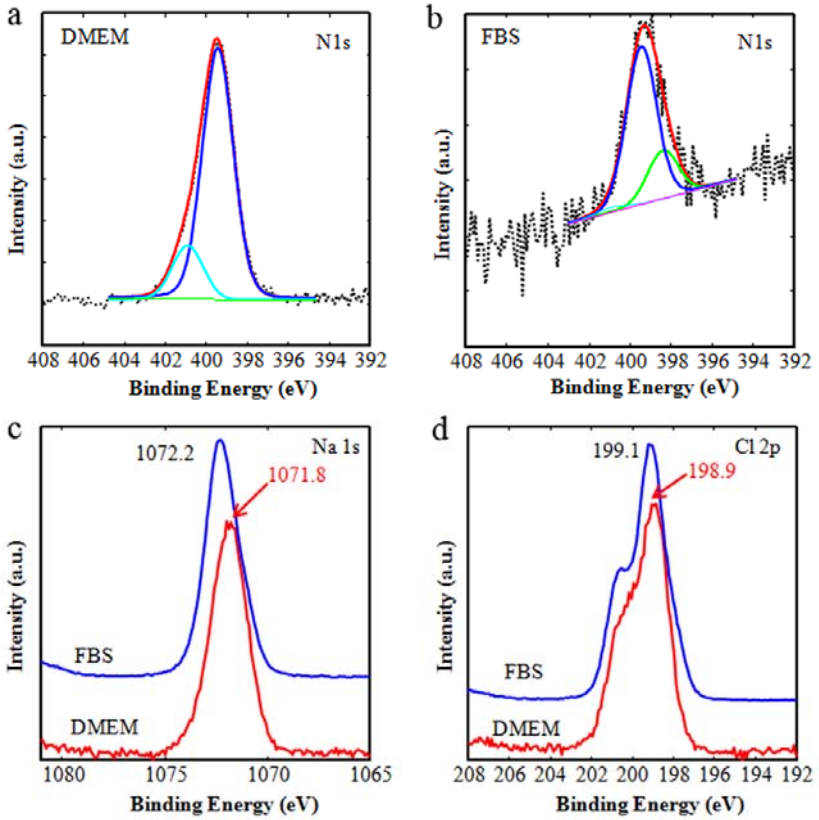

Figure S15. XPS characterization of pristine DMEM and FBS. (a) N1s curve fit measured from DMEM sample; (b) N1s curve fit measured from FBS sample; (c) Na1s spectra of DMEM and FBS; (d) Cl2p spectra of DMEM and FBS.

Table S1. Curve fitting of N1s XPS spectra of different samples

Sample	Band	Position	%Gauss
DMEM	1	399.40	88
	2	400.87	100
FBS	1	398.37	100
	2	**399.41**	91
	3	400.90	100
ZnO+DMEM[1]	1	399.48	100
	2	400.90	100
ZnO+DMEM+FBS[2]	1	398.37	100
	2	**399.76**	100
	3	400.90	100
CuO+DMEM+FBS[3]	1	398.37	100
	2	**399.70**	100
	3	400.90	100

[1] ZnO+DMEM represents that ZnO NPs were dispersed in DMEM.

[2] ZnO+DMEM+PBS represents that ZnO NPs were dispersed in DMEM with FBS.

[3] CuO+DMEM+PBS represents that CuO NPs were dispersed in DMEM with FBS.

Dulbecco's Modified Eagle Medium (Invitrogen) -- **DMEM**

Catalog Number：11885092

COMPONENTS	Molecular Weight	Concentration (mg/L)	mM
Amino Acids			
Glycine	75	30	0.4
L-Arginine hydrochloride	211	84	0.398
L-Cystine 2HCl	313	63	0.201
L-Glutamine	146	584	4
L-Histidine hydrochloride-H2O	210	42	0.2
L-Isoleucine	131	105	0.802
L-Leucine	131	105	0.802
L-Lysine hydrochloride	183	146	0.798
L-Methionine	149	30	0.201
L-Phenylalanine	165	66	0.4
L-Serine	105	42	0.4
L-Threonine	119	95	0.798
L-Tryptophan	204	16	0.0784
L-Tyrosine disodium salt dihydrate	261	104	0.398
L-Valine	117	94	0.803
Vitamins			
Choline chloride	140	4	0.0286
D-Calcium pantothenate	477	4	0.00839
Folic Acid	441	4	0.00907
Niacinamide	122	4	0.0328
Pyridoxine hydrochloride	204	4	0.0196
Riboflavin	376	0.4	0.00106
Thiamine hydrochloride	337	4	0.0119
i-Inositol	180	7.2	0.04

Inorganic Salts			
Calcium Chloride (CaCl2) (anhyd.)	111	200	1.8
Ferric Nitrate (Fe(NO3)3"9H2O)	404	0.1	0.000248
Magnesium Sulfate (MgSO4) (anhyd.)	120	97.67	0.814
Potassium Chloride (KCl)	75	400	5.33
Sodium Bicarbonate (NaHCO3)	84	3700	44.05
Sodium Chloride (NaCl)	58	6400	110.34
Sodium Phosphate monobasic (NaH2PO4-H2O)	138	125	0.906
Other Components			
D-Glucose (Dextrose)	180	1000	5.56
Phenol Red	376.4	15	0.0399
Sodium Pyruvate	110	110	1