Supplementary Information

Enhanced Biostability and Cellular Uptake of Zinc Oxide Nanocrystals Shielded with Phospholipid Bilayer

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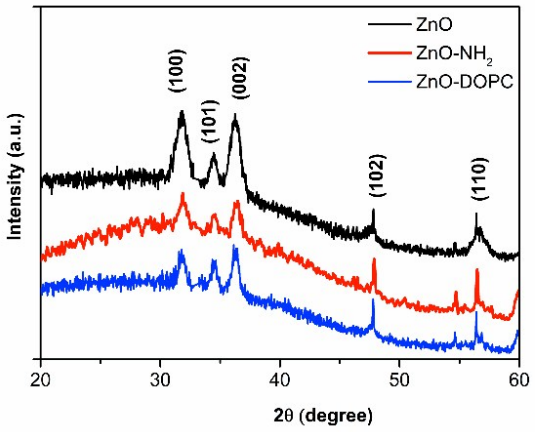

Figure S1. Representative X-Ray diffractogram of the ZnO nanocrystals: pristine (black curve), with amino-propyl functional groups (red curve) and coupled with DOPC lipids (blue curve). Non-indexed peaks derive from the silicon wafer used as substrates.

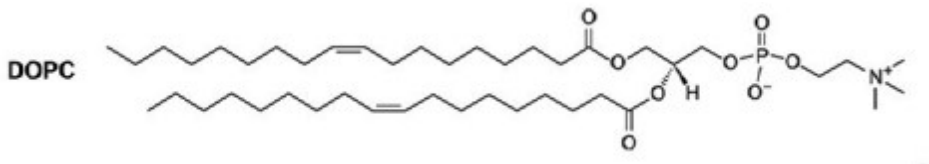

Figure S2. The DOPC phospholipid chemical structure.

Formation of the ZnO-DOPC hybrid construct

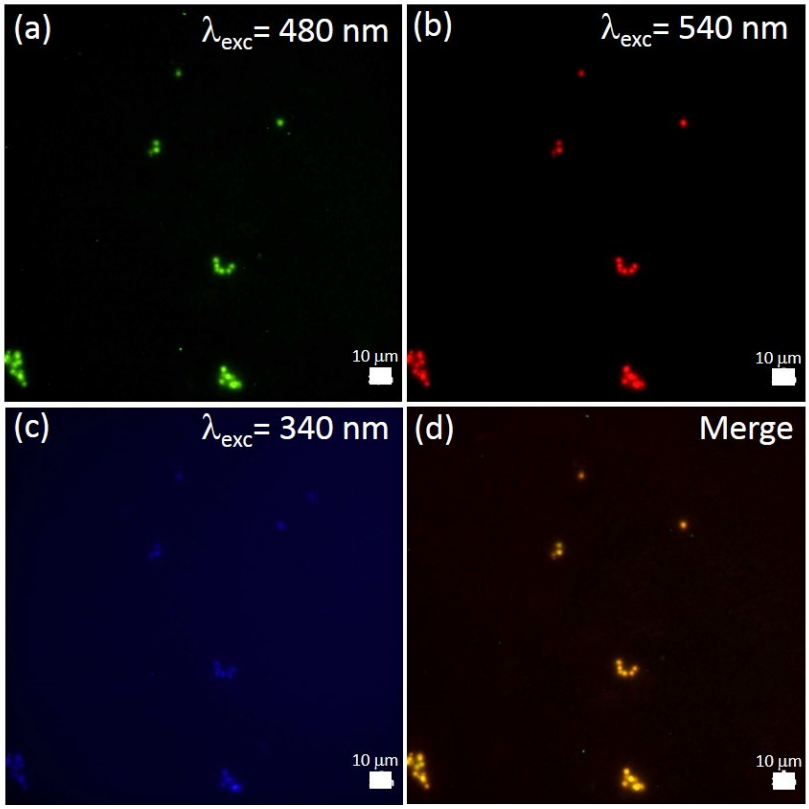

Figure S3. Wide-Field fluorescence microscopy image of the (a) green channel, with the lipid shell labeled by Bodipy, (b) red channel, with the ZnO-NH_2 labeled with Atto550, (c) self-fluorescence of the ZnO NCs exited at 340 nm, (d) co-localization image of the all merged channels.

Composition of Simulated Body Fluid (SBF) and complete Eagle's Minimum Essential Medium (EMEM)

Table S.1. Concentration (mM) of the ions present in Simulated Body Fluid (SBF) and in human plasma [1]

Ions	SBF composition (mM)	Human Plasma Composition (mM)
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	148.8	103.0
HCO_3^-	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

Table S.2. Main components constituting the cell culture media (EMEM, from Sigma Aldrich, product numer: M4655) [2]. In addition to these components, plasma proteins, hormons, vitamins, salts and electrolytes derived from the addition of 10% Fetal Bovine Serum (Sigma Aldrich) should be considered.

EMEM Composition	**Concentration (g/L)**
$CaCl_2$	0.265
$MgSO_4$ (anidro)	0.09767
KCl	0.4
$NaHCO_3$	2.2
NaCl	6.8
NaH_2PO_4 (anidro)	0.122
Amminoacidi tot.	0.8707
Vitamine tot.	0.0081
D-Glucosio	1.0

Table S.3. EDS analysis of the three samples, pristine ZnO, ZnO-NH_2, and ZnO-DOPC after 1 hour, 72 hours and 25 days of biostability assays in SBF solution, reported in % atom.

Element	Before			1h			72h			25d		
	ZnO	ZnO-NH_2	ZnO-DOPC	ZnO	ZnO-NH_2	ZnO-DOPC	ZnO	ZnO-NH_2	ZnO-DOPC	ZnO	ZnO-NH_2	ZnO-DOPC
Zn	33.88	24.24	13.05	24.14	22.83	22.37	26.95	29.91	25.41	22.73	20.51	22.31
O	51.53	41.3	33.55	43.85	46.56	50.11	47.68	45.03	51.74	41.20	32.23	48.77
C	3.73	28.6	50.01	24.47	23.54	16.46	16.84	18.34	8.72	26.52	42.33	18.31
Na	10.86	6.17	2.31	6.04	7.09	7,68	6.47	5.25	5.59	7.13	3.97	5.97
P	-	-	1.09	1.42	-	2.99	1.93	1.48	5.98	2.27	0.97	4.03
Cl	-	-	-	0.10	-	-	-	-	0.29	-	-	-
Ca	-	-	-	-	-	0.40	0.13	-	2.05	0.16	-	0.60

Table S.4. EDS analysis of the three samples, pristine ZnO, ZnO-NH_2, and ZnO-DOPC after 1 hour, 72 hours and 25 days of biostability assays in EMEM solution, reported in % atom.

Element	Before			1h			72h			25d		
	ZnO	ZnO-NH_2	ZnO-DOPC	ZnO	ZnO-NH_2	ZnO-DOPC	ZnO	ZnO-NH_2	ZnO-DOPC	ZnO	ZnO-NH_2	ZnO-DOPC
Zn	33.88	24.24	13.05	18.47	16.02	27.71	22.80	33.12	3.86	7.33	8.48	3.99
O	51.53	41.3	33.55	41.69	39.51	42.22	41.41	44.07	37.25	23.15	21.40	19.12
C	3.73	28.6	50.01	39.42	41.52	23.77	31.43	21.15	49.55	66.63	67.97	74.06
Na	10.86	6.17	2.31	-	2.68	3.25	2.42	-	0,91	1.43	1.85	1.44
P	-	-	1.09	-	-	2.21	1.46	1.23	4.14	0.59	0.42	0.74
Cl	-	-	-	-	-	-	-	-	-	-	0.04	0.05
Ca	-	-	-	0.43	-	0.83	0.49	0.44	3.93	0.50	0.37	0.35
S	-	-	-	-	-	-	-	-	0.11	0.36	0.23	0.19
K	-	-	-	-	0.27	-	-	-	-	-	0.03	0.05
Mg	-	-	-	-	-	-	-	-	0.52	-	-	-

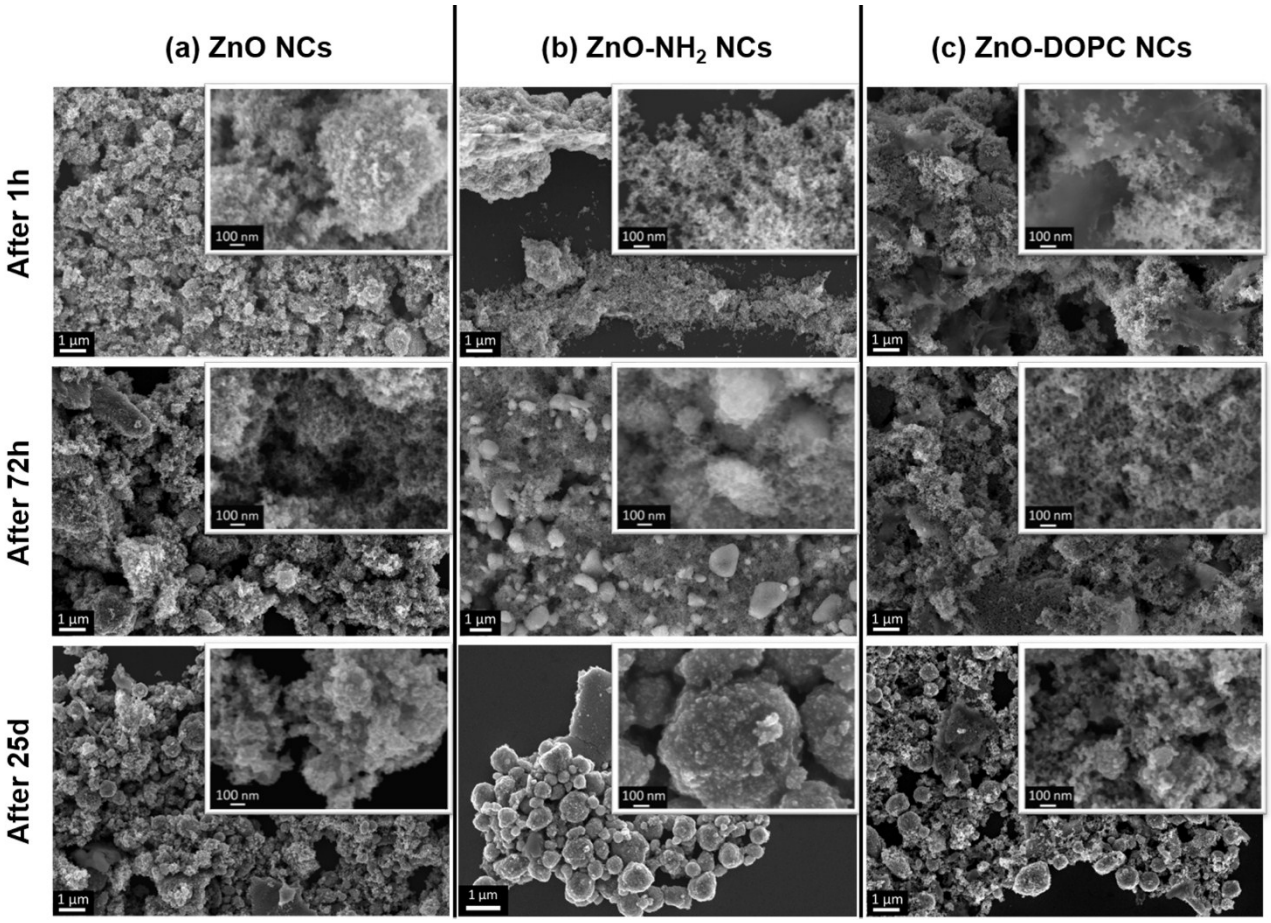

Figure S4. FESEM images of the biostability behavior of the three ZnO nanocrystals: (a) pristine ZnO, (b) amine-functionalized ZnO, (c) lipid-shell functionalized ZnO NCs, after different assay times (1 h, 72 h and 25 days) in SBF.

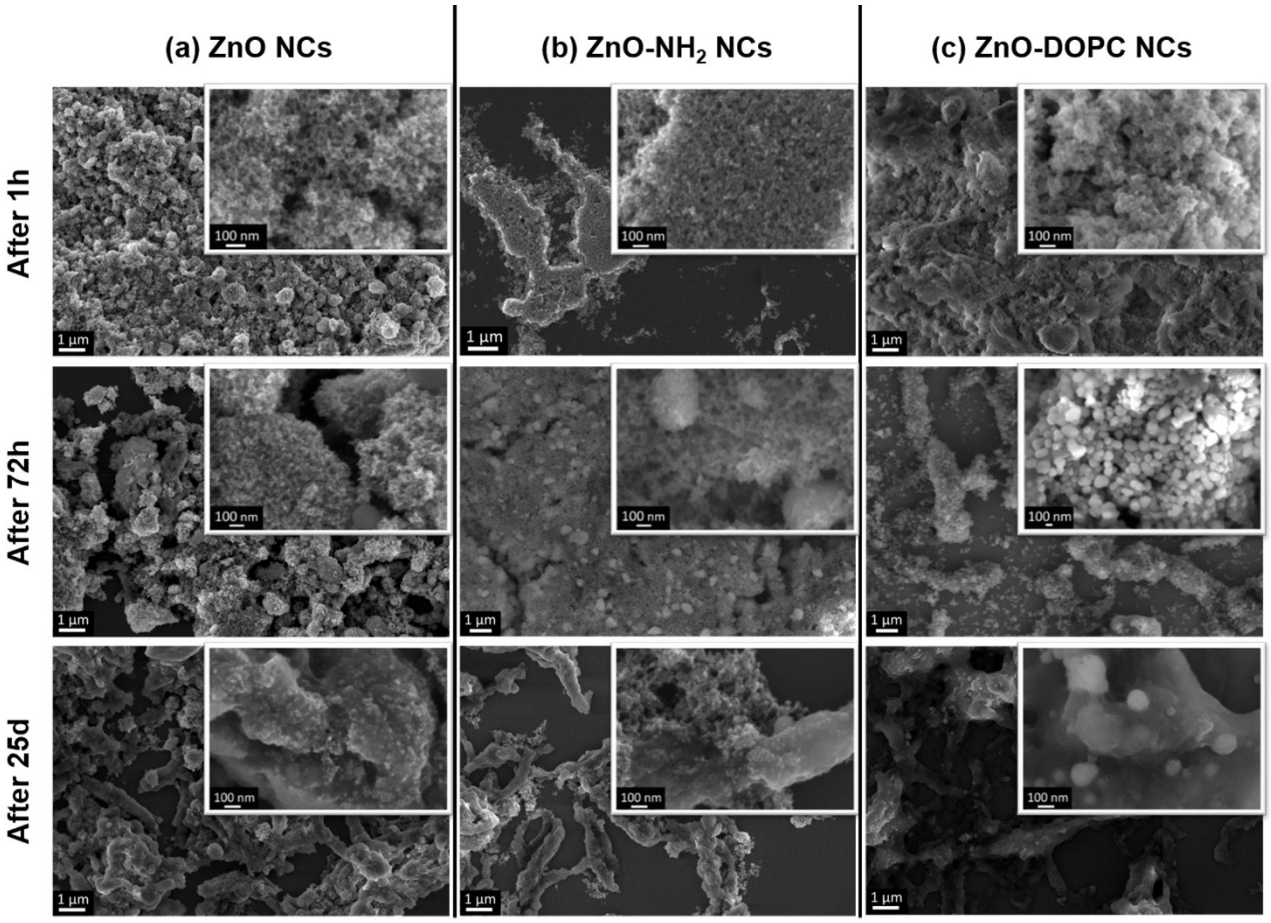

Figure S5. FESEM images of the biostability behavior of the three ZnO nanocrystals: (a) pristine ZnO, (b) amine-functionalized ZnO, (c) lipid-shell functionalized ZnO NCs, after different assay times (1 h, 72 h and 25 days) in EMEM.

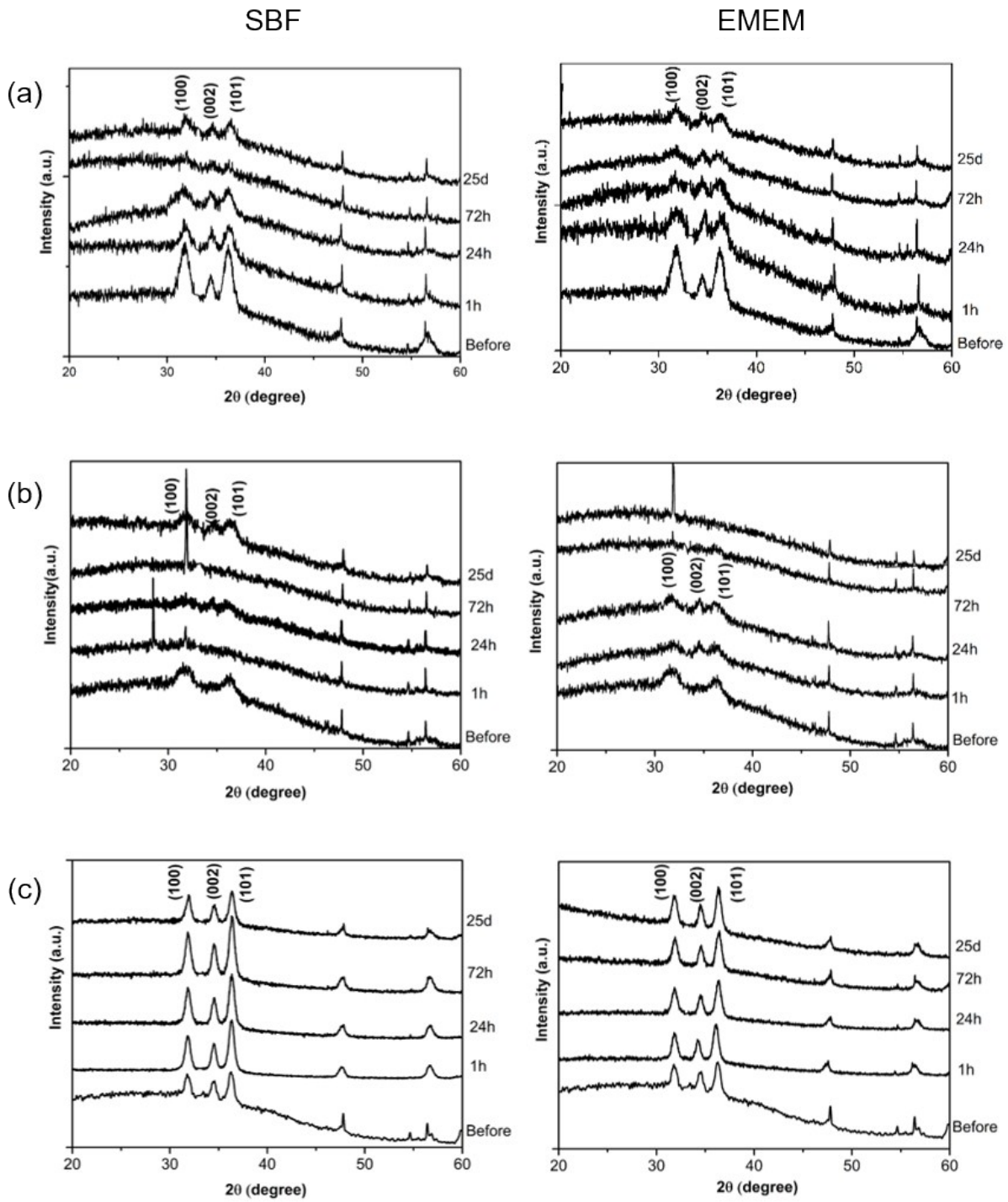

Figure S6. X-ray diffraction patterns of (a) ZnO NCs, (b) ZnO-NH_2 NCs and (c) ZnO-DOPC NCs at different time points during the biostability assays in SBF and EMEM.

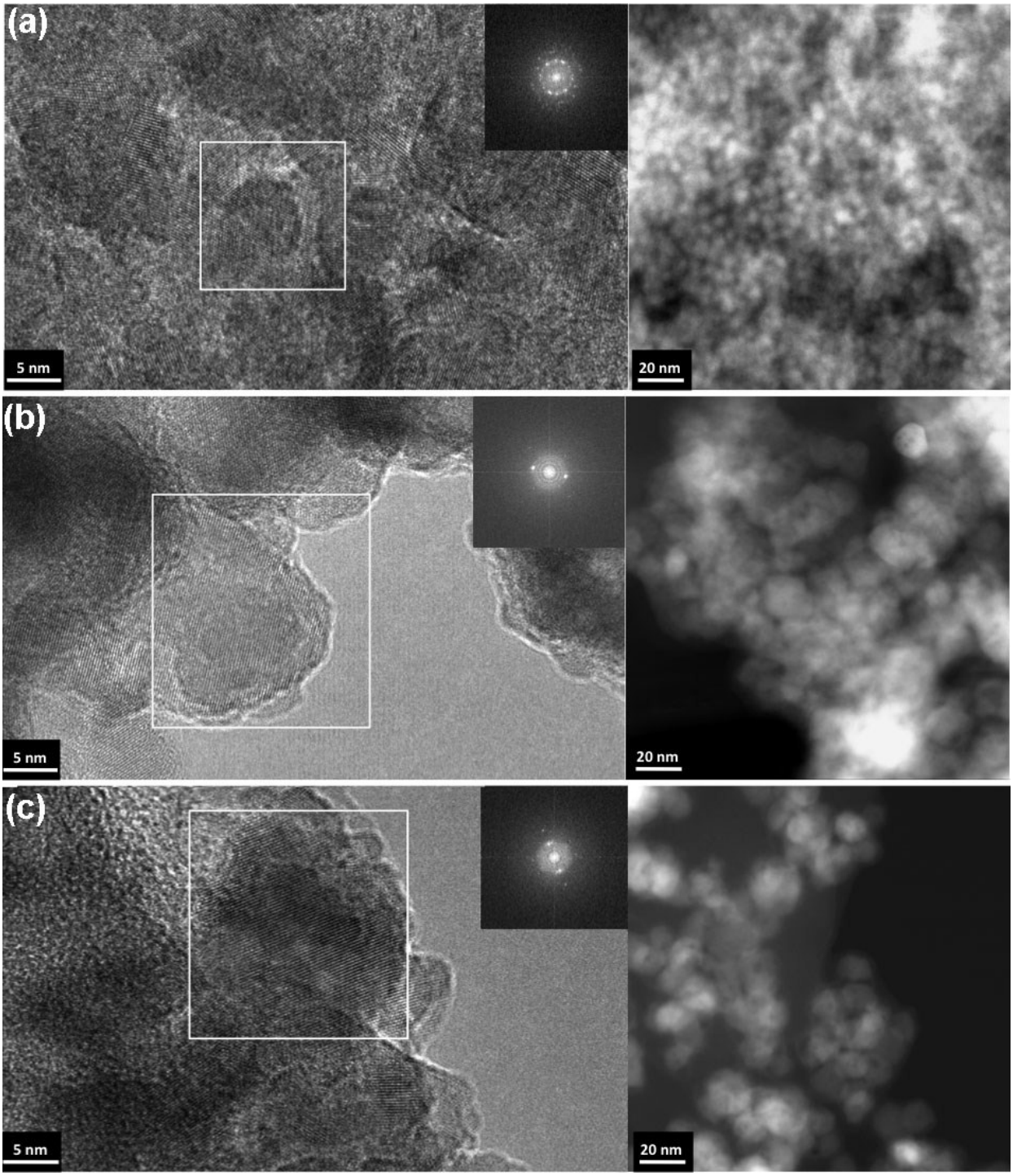

Figure S7. TEM with a FFT in the inset (left) and STEM (right) images of the ZnO-DOPC NCs (a) before, and after 25 days of contact with (b) SBF and (c) EMEM solutions still showing the nano-spherical shape of each NC and a smooth matrix around each of them attributed to the presence of phospholipids.

References

[1] T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi and T. Yamamuro, 1990, Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramic A-W, *J. Biomed. Mater. Res.*, 24, 721-734.
[2] Sigma Aldrich Product Information: http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Formulation/m4655for.pdf.