Supporting Information for New Journal of Chemistry for:

Diosgenin conjugated PCL-MPEG polymeric nanoparticles for co-delivery of anticancer drugs: Design, optimization, *in vitro* drug release and evaluation of anticancer activity

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1. Characterization of PCL-MPEG polymers with diosgenin derivatives

Synthesis of M1-PCL-MPEG

Yield: 94 %, white solid. FT-IR (ATR, cm^{-1}): 3530, 2950, 2850, 1735, 1470, 1240, 1100, 1050, 950, 840. GPC (PS, THF, 0.5 ml/dk): M_n; 15800, M_w; 24020, PDI; 1.52. ^{1}H-NMR (500 MHz, CDCl$_3$, δ, ppm): 5.37 (s, 1H, H$_b$), 4.60 (m, 1H, H$_a$), 4.37 (m, 1H, H$_c$), 4.24 (t, 2H, -CH_2OCO-), 4.06 (t, (CH_2O(C=O) PCL), 3.65 (m, 4H, -O(CH_2CH_2O)-), 3.36 (s, 3H, CH_3-O-), 2.62 (t, 4H, -OCOCH_2CH_2COO-), 2.30 (t, (O(C=O)CH_2) PCL), 1.64 (t, (O(C=O)CH$_2$CH_2CH$_2$CH_2CH$_2$O(C=O))PCL), 1.37 (t, (O(C=O)CH$_2$CH$_2$CH_2CH$_2$CH$_2$O(C=O)) PCL).

Synthesis of M2-PCL-MPEG

Yield: 88 %, white solid. FT-IR (ATR, cm^{-1}): 3530, 2950, 2850, 1735, 1470, 1240, 1100, 1050, 950, 840. GPC (PS, THF, 0.5 ml/dk): M_n; 15500, M_w; 22200, PDI; 1.43. ^{1}H-NMR (500 MHz, CDCl$_3$, δ, ppm): 4.68 (m, 2H, H$_a$, H$_b$), 4.38 (m, 1H, H$_c$), 4.23 (t, 2H, -CH_2CH$_2$OCO-), 4.06 (t, (CH_2O(C=O) PCL), 3.63 (m, 4H, -O(CH_2CH_2O)-), 3.37 (s, 3H, CH_3-O-), 2.62 (t, 4H, -OCOCH_2CH_2COO-), 2.30 (t, (O(C=O)CH_2) PCL), 1.63 (t, (O(C=O)CH$_2$CH_2CH$_2$CH_2CH$_2$O(C=O))PCL), 1.37 (t, (O(C=O)CH$_2$CH$_2$CH_2CH$_2$CH$_2$O(C=O)) PCL).

Synthesis of M3-PCL-MPEG

Yield: 91%, White solid. FT-IR (ATR, cm^{-1}): 3530, 2950, 2850, 1735, 1470, 1240, 1100, 1050, 950, 840. GPC (PS, THF, 0.5 ml/dk): M_n; 15400, M_w; 20800, PDI; 1.35. ^{1}H-NMR (500 MHz, CDCl$_3$, δ, ppm): 5.35 (br, s, 1H, H$_b$), 4.59 (m, 1H, H$_a$), 4.29 (m, 1H, H$_c$), 4.20 (t, 2H, -CH_2CH$_2$OCO-), 4.04 (t, (CH_2O(C=O) PCL), 3.95-3.84 (m, 2H, H$_{ABX}$), 3.63 (m, 4H, -O(CH_2CH_2O)-), 3.37 (s, 3H, CH_3-O-), 3.29 (m, 1H, H$_d$), 2.42 (t, 4H, -OCOCH_2CH_2COO-), 2.30 (t, (O(C=O)CH_2) PCL), 1.63 (t, (O(C=O)CH$_2$CH_2CH$_2$CH_2CH$_2$O(C=O)) PCL), 1.37 (t, (O(C=O)CH$_2$CH$_2$CH_2CH$_2$CH$_2$O(C=O)) PCL).
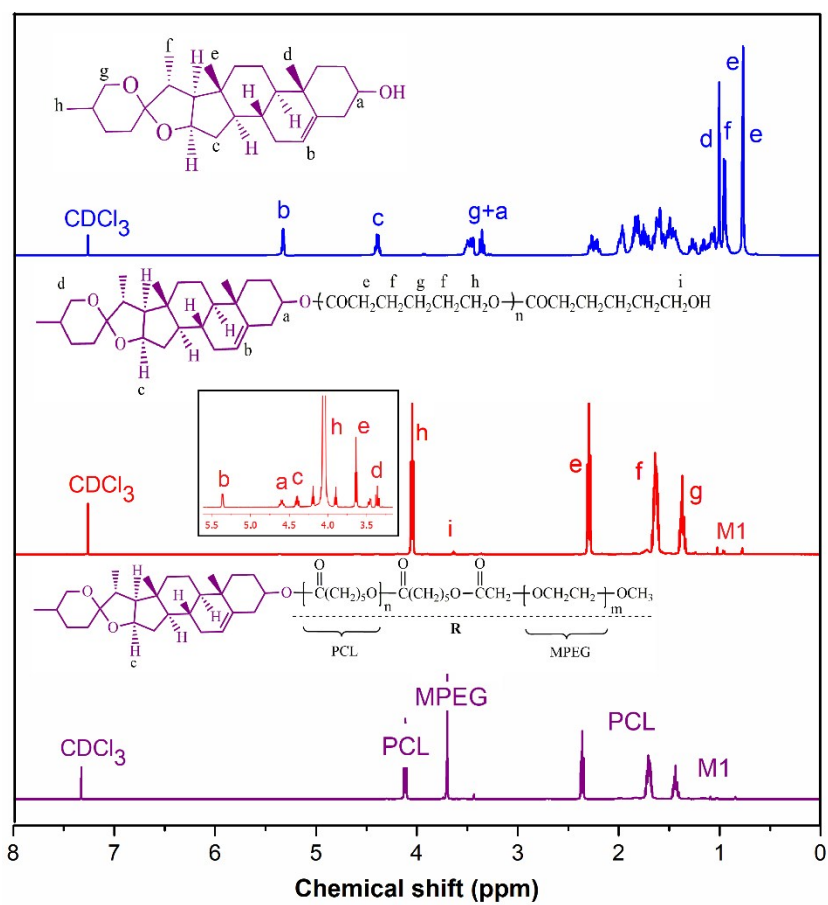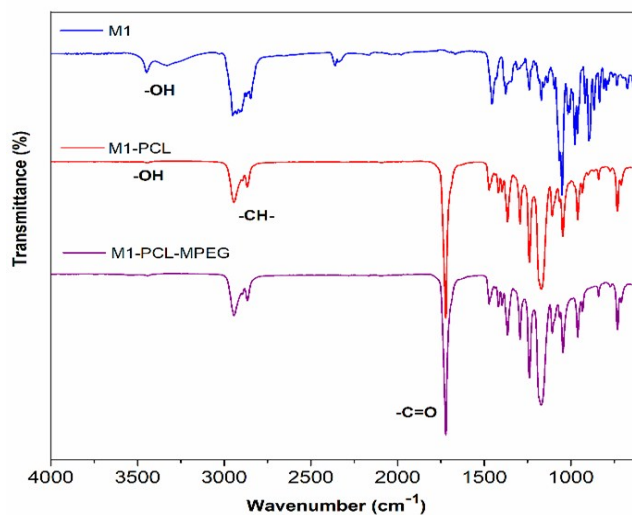
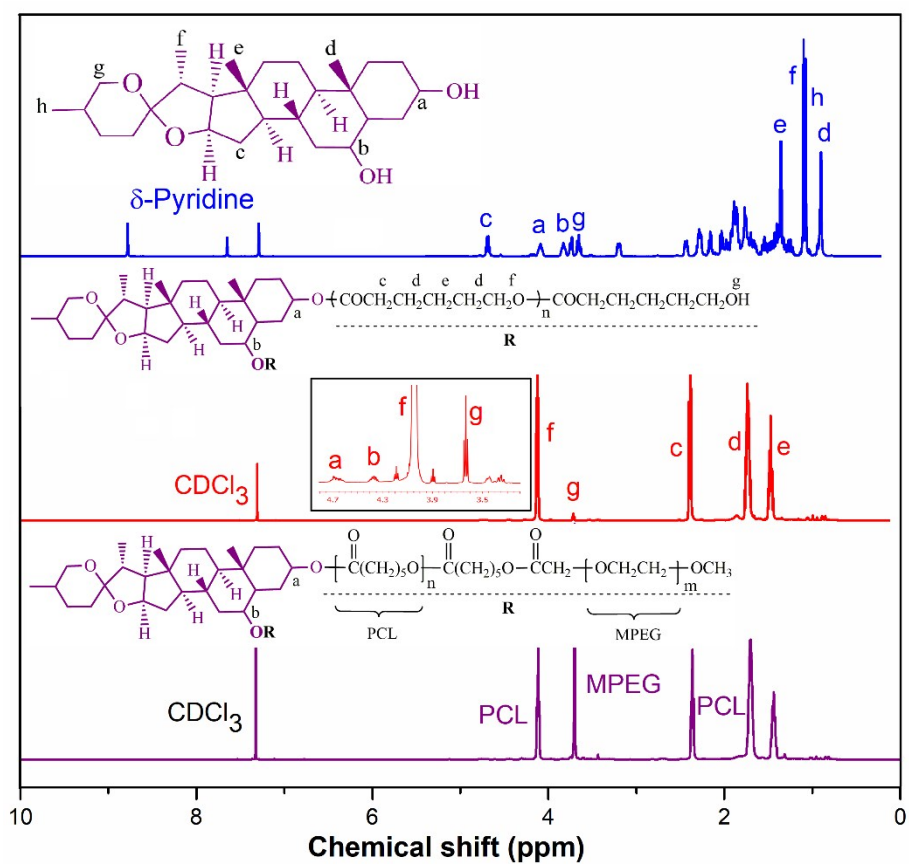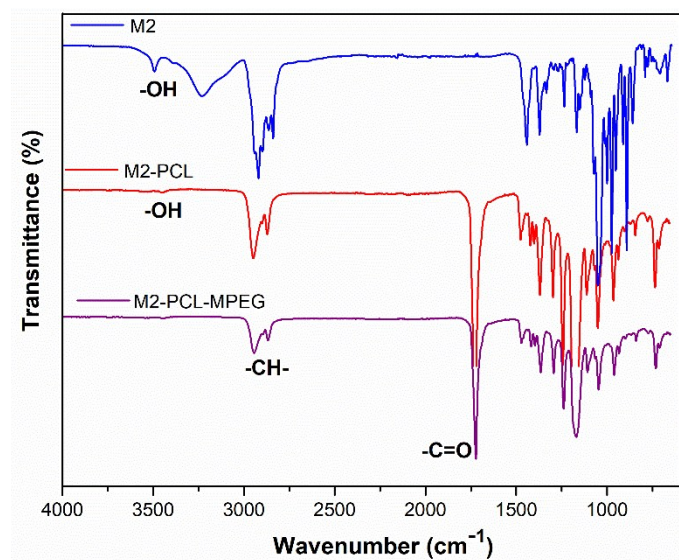Figure S1 FTIR (up) and ^{1}H-NMR (down) spectra of M1-PCL-MPEG copolymers.

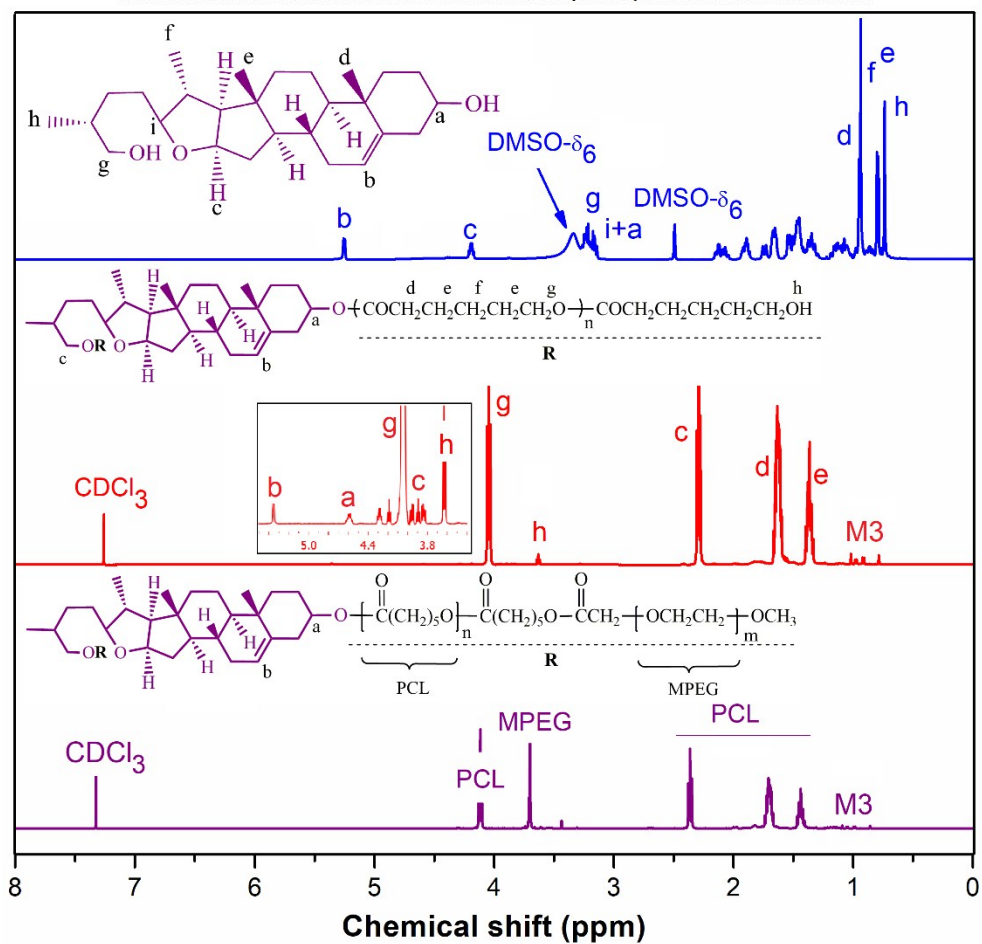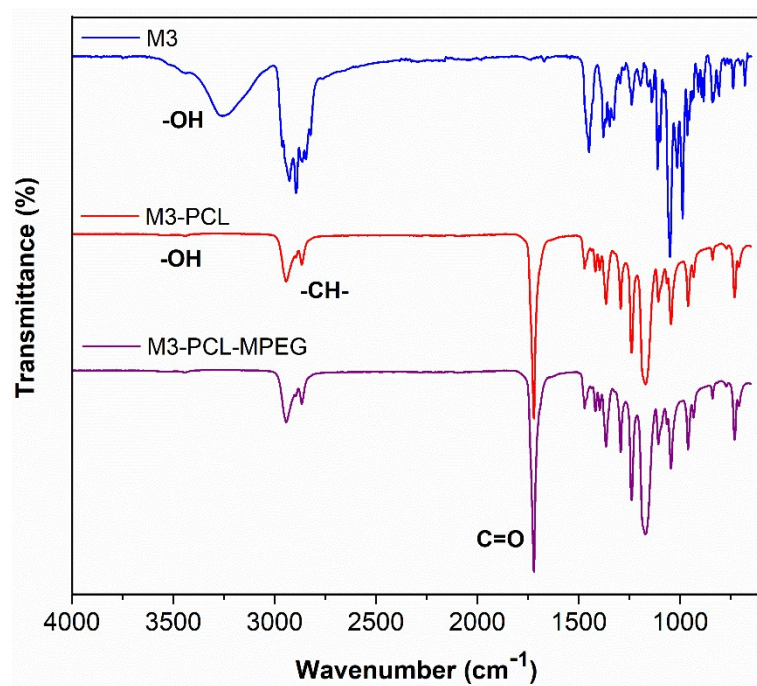Figure S2 FTIR (up) and ^{1}H-NMR (down) spectra of M2-PCL-MPEG copolymers.

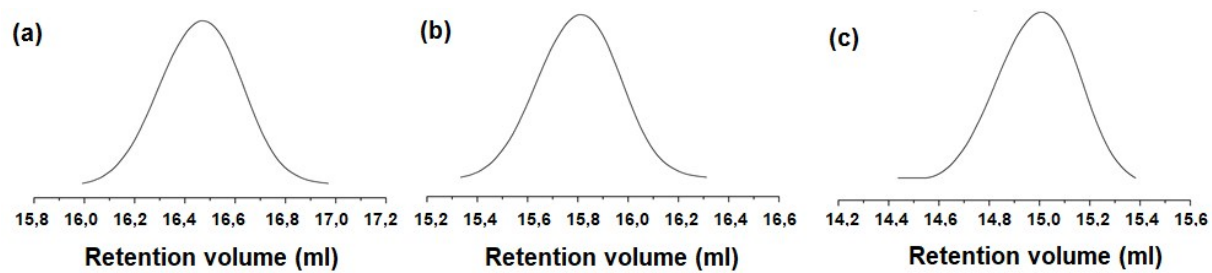Figure S3 FTIR (up) and ^{1}H-NMR (down) spectra of M3-PCL-MPEG copolymers.

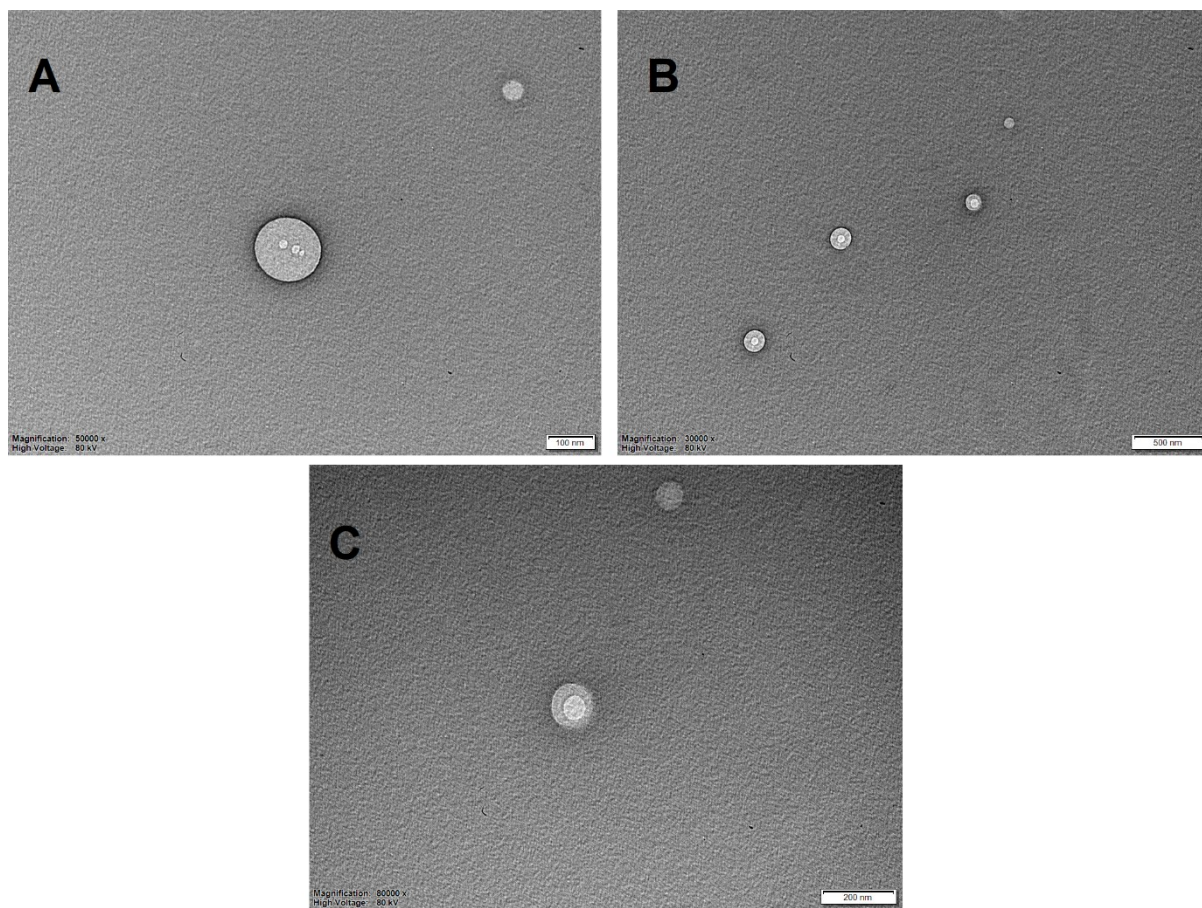Figure S4 GPC Chromatograms of a) mPEG-COOH b) M4-PCL c) M4-PCL-MPEG.

Figure S5 TEM images of a) M1-ITB-NP b) M2-ITB-NP c) M3-ITB-NP.
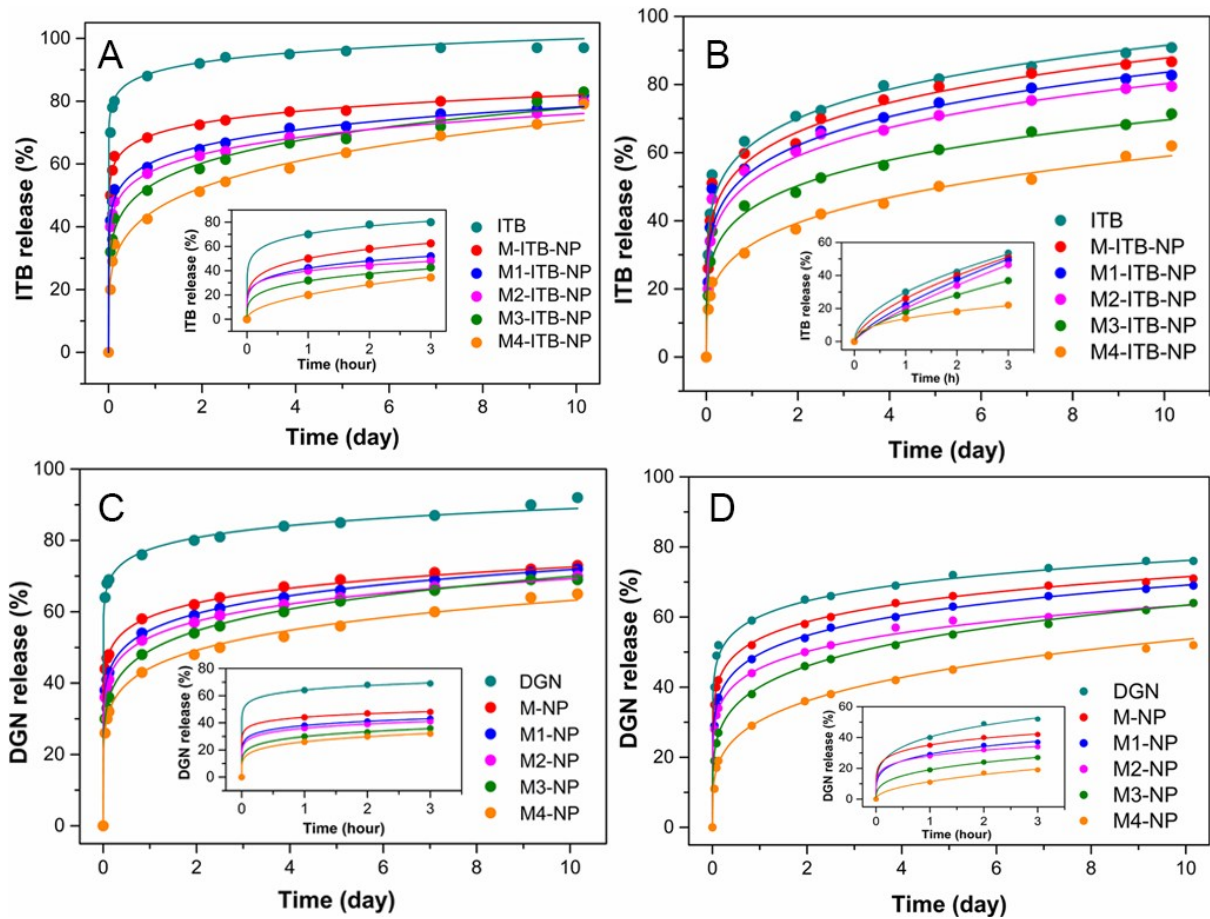
Figure S6 Drug release profiles from NPs and ITB-loaded NPs (A: ITB pH: 3.5, B: ITB pH: 7.4, C: DGN pH: 3.5, and DGN pH: 7.4).
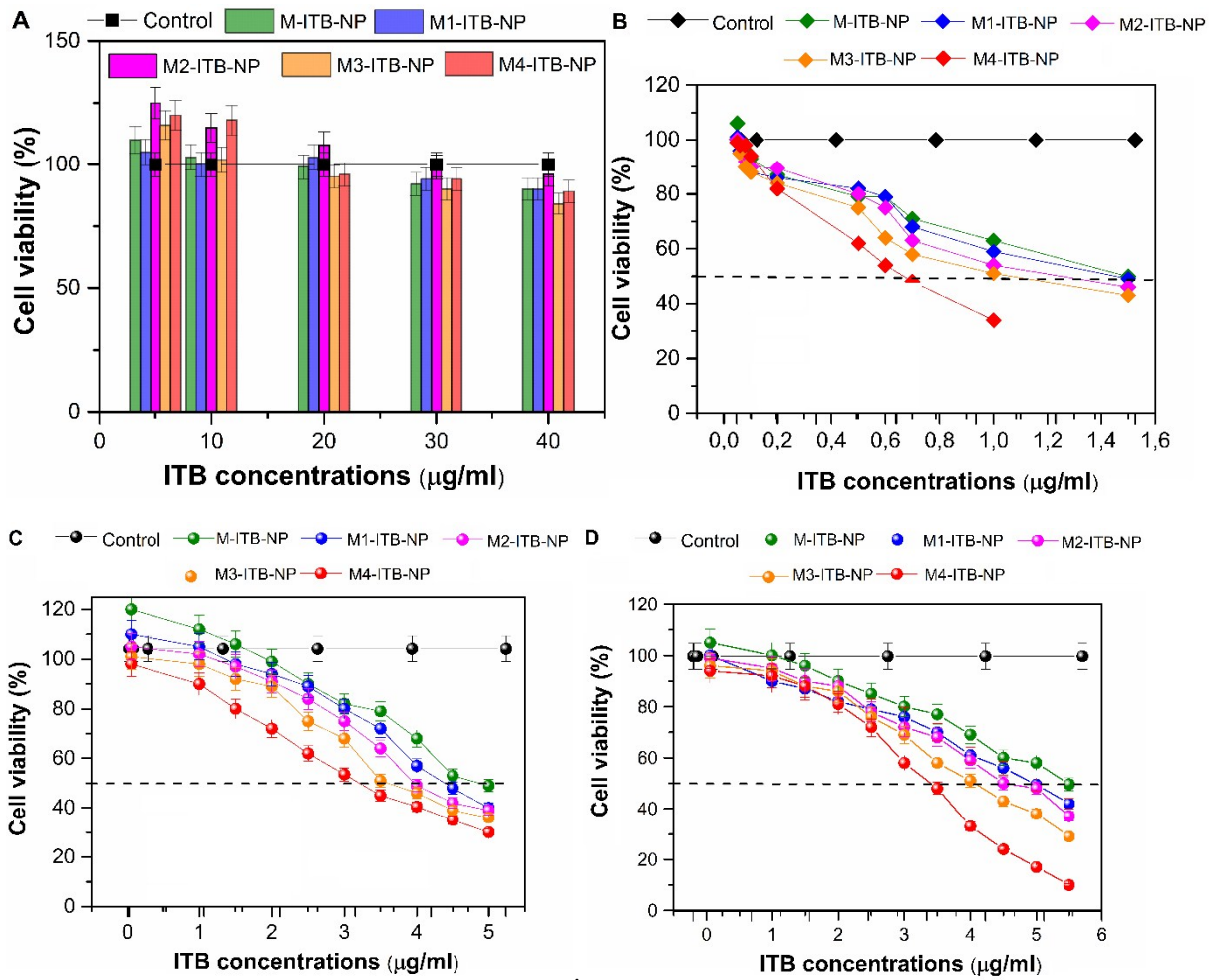
Figure S7 Cell viability of ITB loaded NPs (A: L929, B: MCF-7, C: SAOS-2, and D: K562).

Table S1. ANOVA of formulations influencing the cell viability.

Cell Type	Formulation	ANOVA results				
		Sum of Squares	Mean Square	F value	*P*-value[*]	Adj. R-Squared
MCF-7	ITB	1973.80	493.45	1788.64	<0.0001	0.996
	DGN	7513.82	1878.45	20208.96	<0.0001	0.998
	M4-NP	5598.53	1399.63	9537.75	0	0.998
	M4-ITB-NP	5499.21	1374.80	136.40	<0.0001	0.939
SAOS-2	ITB	3596.13	899.03	1163.46	<0.0001	0.991
	DGN	3598.22	899.55	2535.43	<0.0001	0.988
	M4-NP	4799.26	1199.81	13070.05	<0.0001	0.998
	M4-ITB-NP	4799.41	1199.85	16433.99	<0.0001	0.998
K562	ITB	3996.06	999.01	1524.33	<0.0001	0.995
	DGN	3999.78	999.94	27817.71	<0.0001	0.999
	M4-NP	5199.23	1299.80	15212.21	0	0.997
	M4-ITB-NP	5195.34	1298.83	2508.73	<0.0001	0.997

* *P*-values < 0.05 were considered as statistically significant.