Supporting Information

The influence of graphene oxide – Fe_3O_4 differently conjugated with 10-hydroxycampthotecin and rotating magnetic field on adenocarcinoma cells

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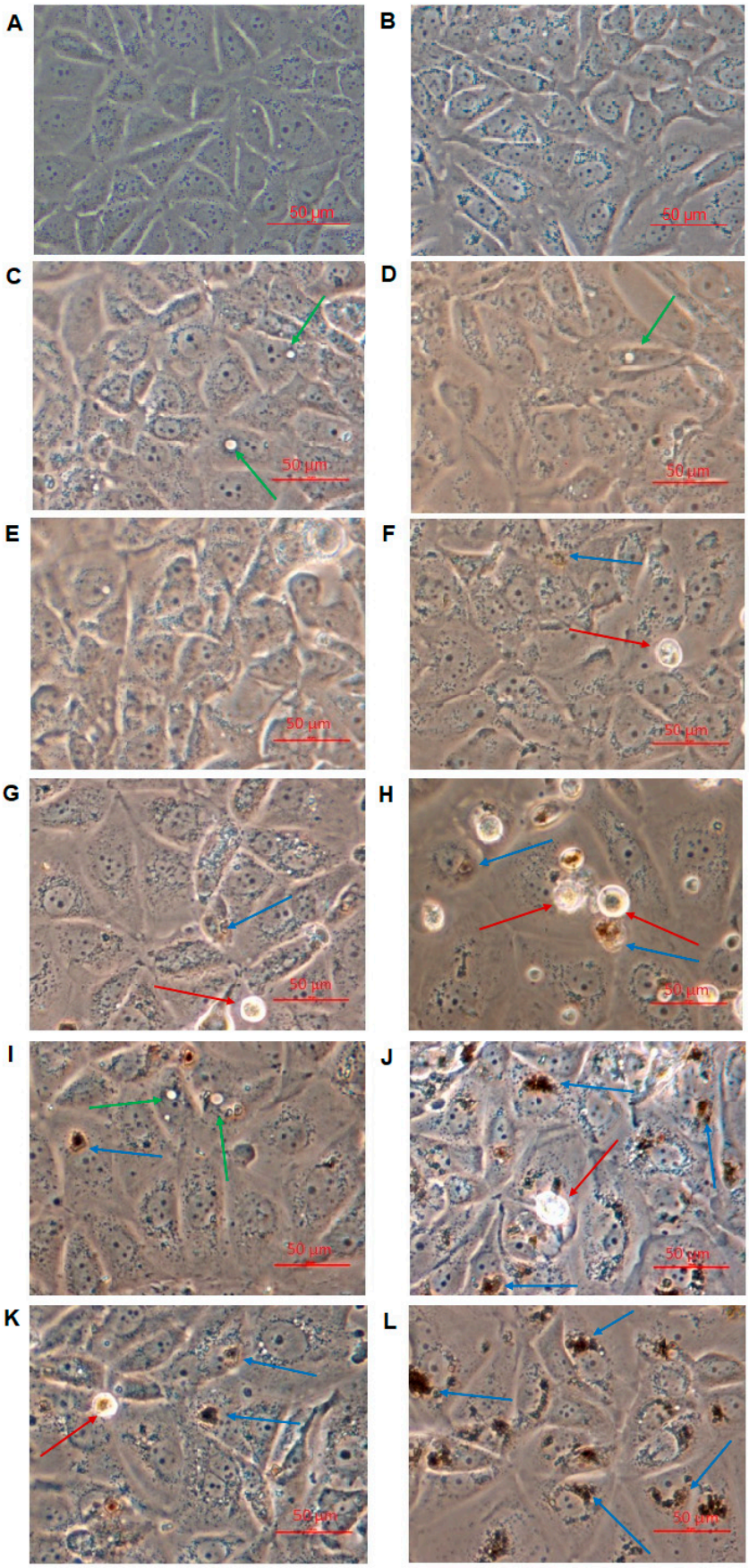

Figure S1. MCF-7 cell line morphology after 8 hours incubation in rotating magnetic field in (a-b) control culture; (c) cultures incubated with c-GO-Fe_3O_4-HCPT and (d) with nc-GO-Fe_3O_4-HCPT at concentration 3.125 µg·ml^{-1}; (e) MCF-7 cells exposed to c-GO-Fe_3O_4-HCPT and (f) to nc-GO-Fe_3O_4-

HCPT at concentration 6.25 $\mu g \cdot ml^{-1}$; (g) MCF-7 cultures incubated with c-GO-Fe_3O_4-HCPT and (h) with nc-GO-Fe_3O_4-HCPT at concentration 12.5 $\mu g \cdot ml^{-1}$; (i) cells exposed to c-GO-Fe_3O_4-HCPT and (j) to nc-GO-Fe_3O_4-HCPT at concentration 25.0 $\mu g \cdot ml^{-1}$; (k) cultures incubated with c-GO-Fe_3O_4-HCPT and (l) with nc-GO-Fe_3O_4-HCPT at concentration 50.0 $\mu g \cdot ml^{-1}$.
Green arrows indicate vacuoles; blue arrows show shrunken cells; red arrows indicate nanomaterials accumulated within cells or aggregates.

Table S1. Statistically significant differences between means obtained from MCF-7 cell cultures incubated with graphene oxide-Fe_3O_4 analyzed using WST-1, LDH and NR assays (small letters present differences considered as significant at a level of $p<0.05$).

	Concentration (μg/mL)																	
	0			3.125			6.25			12.5			25.0			50.0		
	a			b			c			d			e			f		
	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR
GO-Fe_3O_4	c	d	b, c, d, e, f	c, e, f	d	a	a, b, d, e, f	-	a	c, e, f	a, b	a	b, c, d	-	a	b, c, d	-	a

Table S2. Statistically significant differences between means obtained from MCF-7 cell cultures incubated with different concentration of hydroxycamptothecin analyzed using WST-1, LDH and NR assays (small letters present differences considered as significant at a level of $p<0.05$).

	Concentration (µg/mL)																				
	0			DMSO			3.125			6.25			12.5			25.0			50.0		
	a			b			c			d			e			f			g		
	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR
HCPT	b, c, d, e, f, g	b, c, d, e, f, g	b, c, d, e, f, g	a, c, d, e, f, g	a, c, d, e, f, g	a, c, d, e, f, g	a, b	a, b	a, b, d	a, b	a, b	a, b, c, f, g	a, b	a, b	a, b, c	a, b	a, b	a, b, d	a, b	a, b	a, b, d

Table S3. Statistically significant differences between means of MCF-7 cell controls and experimental samples exposed to different magnetic induction intensity of RMF obtained from WST-1, LDH and NR assays (small letters present differences considered as significant at a level of $p<0.05$).

	Magnetic induction (mT)																				
	0 (without RMF)			1.23			1.57			2.36			3.95			6.58			10.06		
	a			b			c			d			e			f			g		
	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR
8 h exposition	e, f, g	b, c, d, g	a	f, g	a, e, f, g	a	g	a, e, f, g	a	g	a, f, g	a	a	b, c, g	a	a, b	b, c, d, g	a	a, b, c, d	a, b, c, d, e, f	a, b, c, d, e, f
24 h after exposition	e, f, g	g	a	-	g	a	-	g	a	-	g	a	a	g	a	a	g	a	a	a, b, c, d, e, f	a, b, c, d, e, f

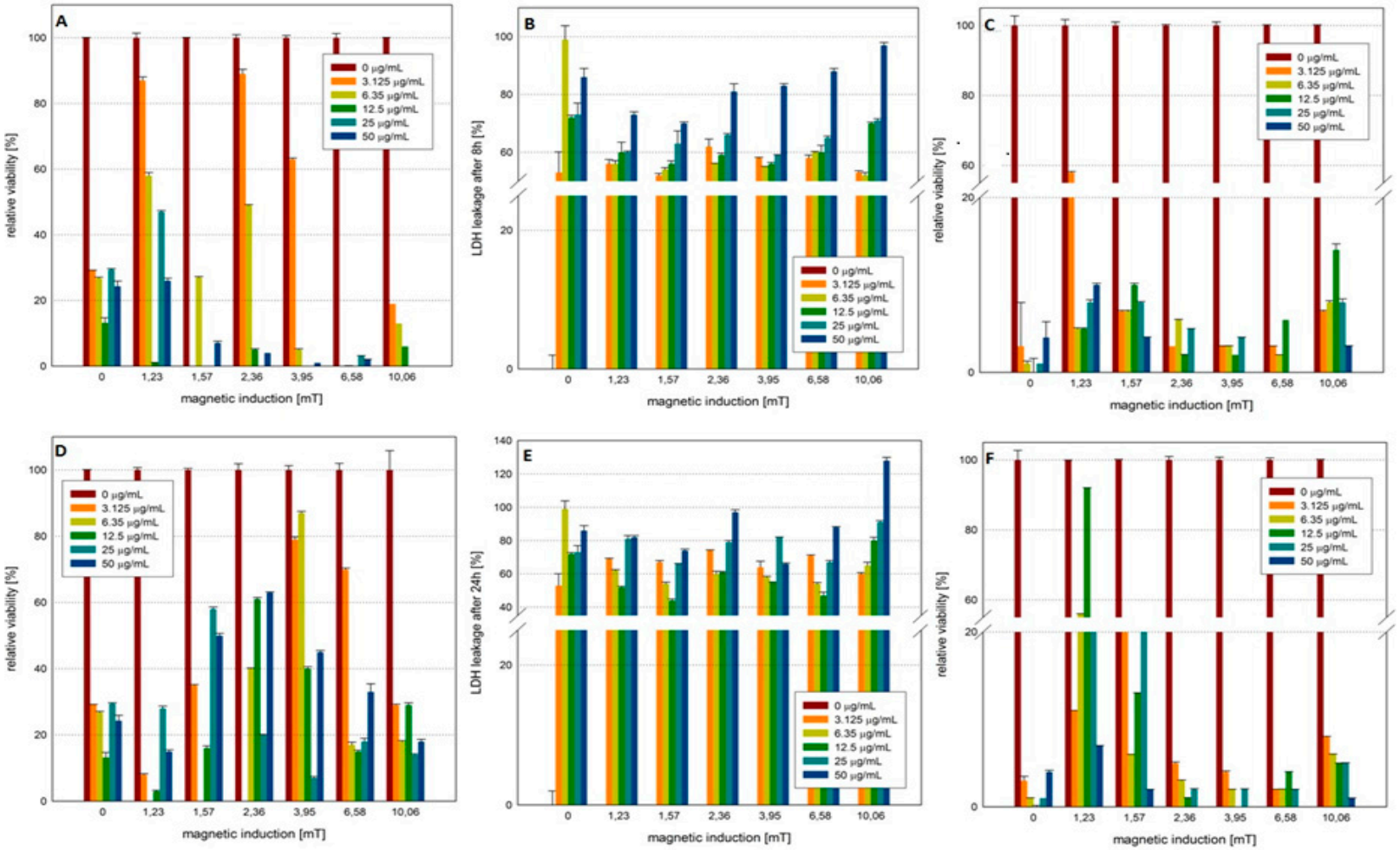

Figure S2. The response of MCF-7 cells to c-GO-Fe_3O_4-HCPT nanomaterial and RMF; (a) relative viability (WST-1 assay results); (b) LDH membrane leakage (LDH assay results); (c) neutral red uptake (NR assay results); (d) relative viability 24-hour after RMF exposure (WST-1 assay); (e) LDH release 24-hour after RMF exposure (LDH assay); (f) NR uptake 24-hour after RMF exposure (p-values<0.05 are considered significant and are represented by small letters, Table S4).

Table S4. Statistically significant differences between means of MCF-7 cell controls and experimental samples exposed to different magnetic induction intensity of RMF obtained from WST-1, LDH and NR assays (small letters present differences considered as significant at a level of $p<0.05$).

Type of nanomaterial	Magnetic induction (mT)																				
	0 (without RMF)			1.23			1.57			2.36			3.95			6.58			10.06		
	a			b			c			d			e			f			g		
	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR
c-GO-Fe_3O_4-CPT 8 h	c, d, f, g	b, c, d, e, f, g	b, c, d, e, f, g	c, d, e, f, g	a	a	a, b, d, e, f, g	a	a	a, b, c, g	a	a	b, c, g	a	a	a, b, c, g	a	a	a, b, c, d, e, f	a	a
c-GO-Fe_3O_4-CPT 24 h	c, f, g	b, c, d, e, f, g	b, c, d, e, f	c, f, g	a, g	a, d, e, f, g	a, b, d, e, f, g	a, g	a, d, e, f, g	c, f, g	a, g	a, b, c, e, f, g	c, f, g	a, g	a, b, c, e, f, g	a, b, c, d, e, g	a, g	a, b, c, d, e	a, b, c, d, e, f	a, b, c, d, e, f	b, c, d, e
nc-GO-Fe_3O_4-CPT 8 h	b, c, d, e, f, g	b, c, d, e, f, g	b, c, d, e, f, g	a, c, d, e, f, g	a	a	a, b, f, g	a	a	a, b, f, g	a	a	a, b, f, g	a	a	a, b, c, d, e, g	a	a	a, b, c, d, e, f	a	a
nc-GO-Fe_3O_4-CPT 24 h	b, c, d, e, f, g	b, c, d, e, f, g	d, e, f, g	a, g	a	d, e, f, g	a, g	a, d, e, f	d, e, f, g	a, g	a, c	a, b, c, e	a, g	a, c	a, b, c, d, f	a, g	a, b, c, e	a, c	a, b, c, d, e, f,	a	a, b, c

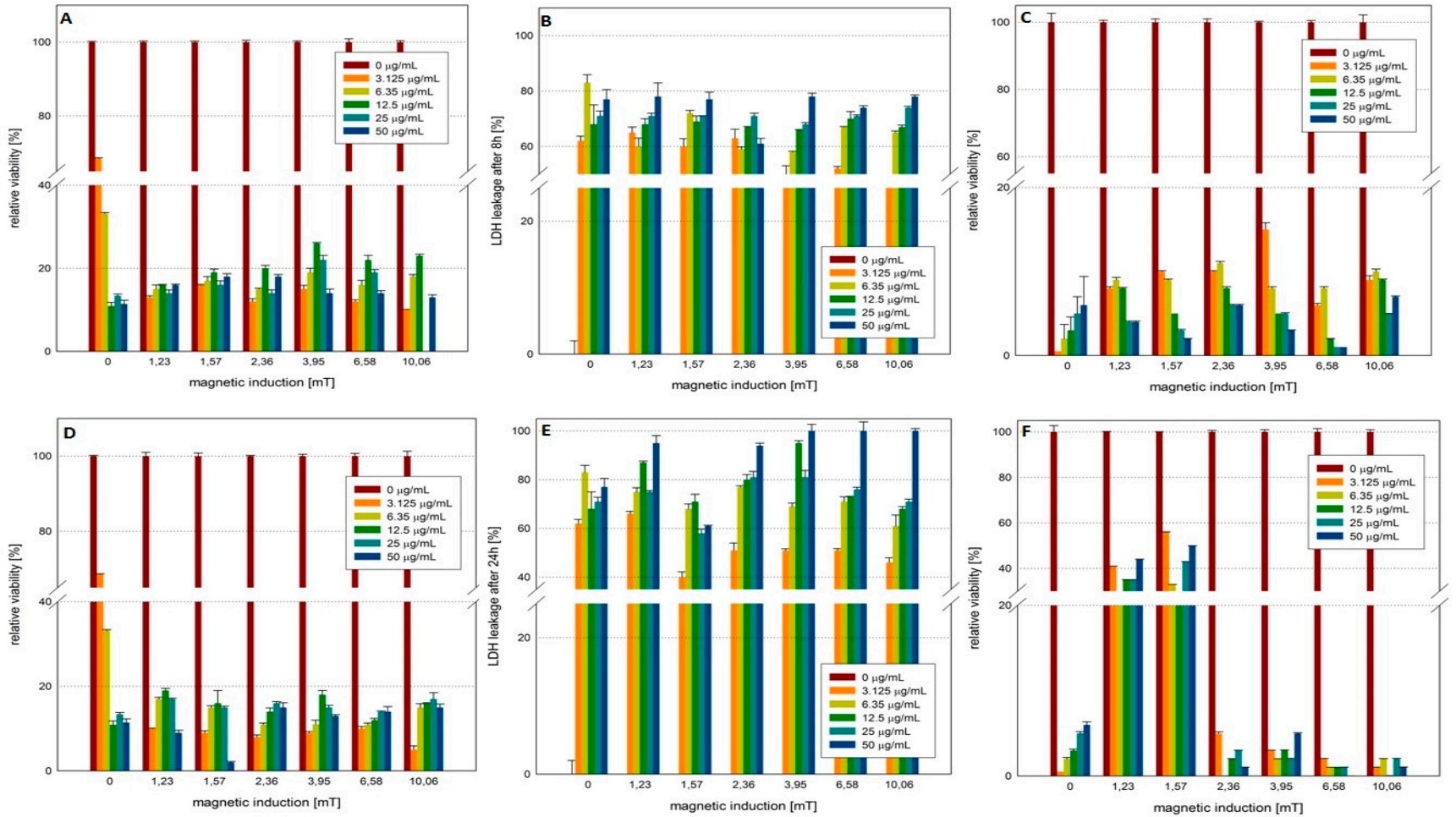

Figure S3. The response of MCF-7 to nc-GO-Fe_3O_4-HCPT nanomaterial and RMF; (a) relative viability (WST-1 assay results); (b) LDH membrane leakage (LDH assay results); (c) neutral red uptake (NR assay results); (d) relative viability 24-hour after RMF exposure (WST-1 assay results); (e) LDH release 24-hour after RMF exposure (LDH assay results); (f) NR uptake assay 24-hour after RMF exposure (p-values<0.05 are considered significant and are represented by small letters, Table S5).

Table S5. Statistically significant differences between means of MCF-7 cell controls and experimental samples exposed to different magnetic induction intensity of RMF obtained from WST-1, LDH and NR assays (small letters present differences considered as significant at a level of $p<0.05$).

Type of nanomaterial	NPs concentration (μg/mL)																	
	0 (without NPs)			3.125			6.25			12.5			25.0			50.0		
	a			b			c			d			e			f		
	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR	WST-1	LDH	NR
c-GO-Fe_3O_4-CPT 8 h	b, c, d, e, f	b, c, d, e, f	b, c, d, e, f	a, c, d, e, f	a, f	a	a, b, d, e	a, f	a	a, b, c, f	a, f	a	a, b, c, f	a	a, f	a, b, d, e	a, b, c, d, e	a
c-GO-Fe_3O_4-CPT 24 h	b, c, d, e, f	b, c, d, e, f	b, c, d, e, f	a, c, d, e, f	a, d, f	a, f	a, b, e	a, b, f	a	a, b, f	a, b, e, f	a	a, b, c, f	a, c, d, f	a	a, b, d, e	a, b, c, d, e	a, b
nc-GO-Fe_3O_4-CPT 8 h	b, c, d, e, f	b, c, d, e, f	b, c, d, e, f	a, c, d	a, d, e, f	a	a, b, e, f	a	a	a, b, e, f	a, b	a	a, c, d	a, b	a	a, c, d	a, b	a
nc-GO-Fe_3O_4-CPT 24 h	b, c, d, e, f	b, c, d, e, f	b, c, d, e, f	a, c, d, e	a, c, d, e, f	a	a, b, f	a, b, f	a	a, b, f	a, b, f	a	a, b, f	a, b, f	a	a, c, d, e	a, b, c, d, e	a